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Annimari Lehtomäki

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ABSTRACT

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The feasibility of utilising energy crops and crop residues in methane production through anaerobic digestion in boreal conditions was evaluated in this thesis. Potential boreal energy crops and crop residues were screened for their suitability for methane production, and the effects of harvest time and storage on the methane potential of crops was evaluated. Co-digestion of energy crops and crop residues with cow manure, as well as digestion of energy crops alone in batch leach bed reactors with and without a second stage upflow anaerobic sludge blanket reactor (UASB) or methanogenic filter (MF) were evaluated. The methane potentials of crops, as determined in laboratory methane potential assays, varied from 0.17 to 0.49 m^3 CH_4 kg^{-1} VS_{added} (volatile solids added) and from 25 to 260 m^3 CH_4 t^{-1} ww (tons of wet weight). Jerusalem artichoke, timothy-clover and reed canary grass gave the highest methane potentials of 2 900–5 400 m^3 CH_4 ha^{-1}, corresponding to a gross energy potential of 28–53 MWh ha^{-1} and 40 000–60 000 km ha^{-1} in passenger car transport. The methane potentials per ww increased with most crops as the crops matured. Ensiling without additives resulted in minor losses (0–13%) in the methane potential of sugar beet tops but more substantial losses (17–39%) in the methane potential of grass, while ensiling with additives was shown to have potential in improving the methane potentials of these substrates by up to 19–22%. In semi-continuously fed laboratory continuously stirred tank reactors (CSTRs) co-digestion of manure and crops was shown feasible with feedstock VS containing up to 40% of crops. The highest specific methane yields of 0.268, 0.229 and 0.213 m^3 CH_4 kg^{-1} VS_{added} in co-digestion of cow manure with grass, sugar beet tops and straw, respectively, were obtained with 30% of crop in the feedstock, corresponding to 85–105% of the methane potential in the substrates as determined by batch assays. Including 30% of crop in the feedstock increased methane production per digester volume by 16–65% above that obtained from digestion of manure alone. In anaerobic digestion of energy crops in batch leach bed reactors, with and without a second stage methanogenic reactor, the highest methane yields were obtained in the two-stage process without pH adjustment. This process was well suited for anaerobic digestion of the highly degradable sugar beet and grass-clover silage, yielding 0.382–0.390 m^3 CH_4 kg^{-1} VS_{added} within the 50–55 day solids retention time, corresponding to 85–105% of the methane potential in the substrates. With the more recalcitrant substrates, first year shoots of willow and clover-free grass silage, the methane yields in this process remained at 59–66% of the methane potential in substrates. Only 20% of the methane potential in grass silage was extracted in the one-stage leach bed process, while up to 98% of the total methane yield in the two-stage process originated from the second stage methanogenic reactor. Liquid and solid residues from digestion of grass-clover silage and sugar beet in two-stage leach bed – MF processes were suitable for incorporation to soil as fertiliser and soil-improvement media, whereas in the solid residue from digestion of willow, cadmium concentration exceeded the limit value for use of digestates as fertiliser in arable land.

Keywords: Anaerobic digestion; biogas; co-digestion; crop residues; CSTR; energy crops; harvest time; leach bed; methane; silage; storage.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is a summary and discussion of the following articles and manuscripts, which are referred to by their Roman numerals I – V in the text.

I Lehtomäki, A., Viinikainen, T. & Rintala, J. Screening boreal energy crops and crop residues for methane biofuel production. Submitted.

II Lehtomäki, A., Ronkainen, O. & Rintala, J. The effect of storage on methane production from energy crops and crop residues. Submitted.

III Lehtomäki, A., Huttunen, S. & Rintala, J. Co-digestion of energy crops and crop residues with cow manure for methane production: Effect of crop to manure ratio. Submitted.

IV Lehtomäki, A., Huttuen, S., Lehtinen, T. & Rintala, J. Anaerobic digestion of grass silage in batch leach bed reactors for methane production. Submitted.

V Lehtomäki, A. & Björnsson, L. 2006. Two-stage anaerobic digestion of energy crops: Methane production, nitrogen mineralisation and heavy metal mobilisation. Environmental Technology 27: 209–218.

ABBREVIATIONS

ADF	acid detergent fibre
ASBR	anaerobic sequencing batch reactor
C_{tot}	total carbon
CFU	colony-forming unit
COD	chemical oxygen demand
CSTR	continuously stirred tank reactor
EU	European Union
FA	formic acid
FW	fresh weight
GC	gas chromatograph
ha	hectare
HPLC	high performance liquid chromatograph
HRT	hydraulic retention time
ICP-MS	inductively coupled plasma mass spectrometer
LAB	lactic acid bacteria
L/S	liquid / solid
$lignin_{AS}$	acid soluble lignin
$lignin_{tot}$	total lignin
MF	methanogenic filter
N_{tot}	total nitrogen
NDF	neutral detergent fibre
NH_4-N	ammonium nitrogen
OLR	organic loading rate
org-N	organic nitrogen
rpm	revolutions per minute
SCOD	soluble chemical oxygen demand
t	metric ton
toe	tons of oil equivalent
TS	total solids
TVFA	total volatile fatty acids
UASB	upflow anaerobic sludge blanket
VFA	volatile fatty acids
VS	volatile solids
ww	wet weight

1 INTRODUCTION

Production of methane-rich biogas through anaerobic digestion of organic materials provides a versatile carrier of renewable energy, as methane can be used in replacement for fossil fuels in both heat and power generation and as a vehicle fuel, thus contributing to cutting down the emissions of greenhouse gases and slowing down the climate change. Methane production through anaerobic digestion has been evaluated as one of the most energy-efficient and environmentally benign ways of producing vehicle biofuel (LBS 2002). The European Union (EU) has set a target of increasing the utilisation of biofuels in vehicles to 5.75% by year 2010 in each member state (European Parliament 2003), while in 2005, the market share of biofuels in Finland was 0.1% (Commission of the European Communities 2005). Methane production from energy crops and crop residues could be an interesting option for increasing the domestic biofuel production, as it has been estimated that within the agricultural sector in the EU, 1 500 million tons (t) of biomass could be anaerobically digested each year, half of this potential accounted for by energy crops (Amon et al. 2001). The total energy content of digestible agricultural waste and landfill gas in the EU has been estimated to exceed 80 million tons of oil equivalents (Mtoe) annually. The annual contribution that could be made by biogas exploitation from livestock production, agro-industrial effluents, sewage treatment and landfill in the EU by 2010 is estimated at 15 Mtoe, while 30 and 45 Mtoe has been estimated achievable annually by 2010 from wood and agricultural residues, and energy crops, respectively, in the EU (European Commission 1997). The crude biogas production in the EU amounted to 4 Mtoe in 2004, while in Finland, the corresponding figure was 0.017 Mtoe, low compared to those in United Kingdom (1.5 Mtoe) and Germany (1.3 Mtoe), for example (EurObser'ER 2005). By the end of 2005, there were approximately 3 000 farm biogas plants in operation in Germany (Weiland 2005), while in Finland, the corresponding figure in the end of 2004 was six (Kuittinen et al. 2005). The biogas potential in Finland has been estimated at 14 TWh not including energy crops (Lampinen 2003). However, the Ministry of Agriculture

has proposed that by 2012 up to 500 000 ha, an area corresponding to about one fourth of all arable land in Finland, could be dedicated to energy crop production (Vainio-Mattila et al. 2005). In Sweden, the corresponding figure has been estimated at 600 000 ha by 2020, yielding 10–20 TWh of energy per year (Herland 2005).

The chain for producing methane through anaerobic digestion from energy crops is presented in Fig. 1, from the production and harvest of crop biomass, to storage and pre-treatment of the biomass, production and utilisation of biogas, storage, post-methanation and post-treatment of the digestate, and finally returning the digestate back to the crop production areas as fertiliser and soil-improvement medium. The various aspects of the production chain are discussed in the following chapters of this thesis.

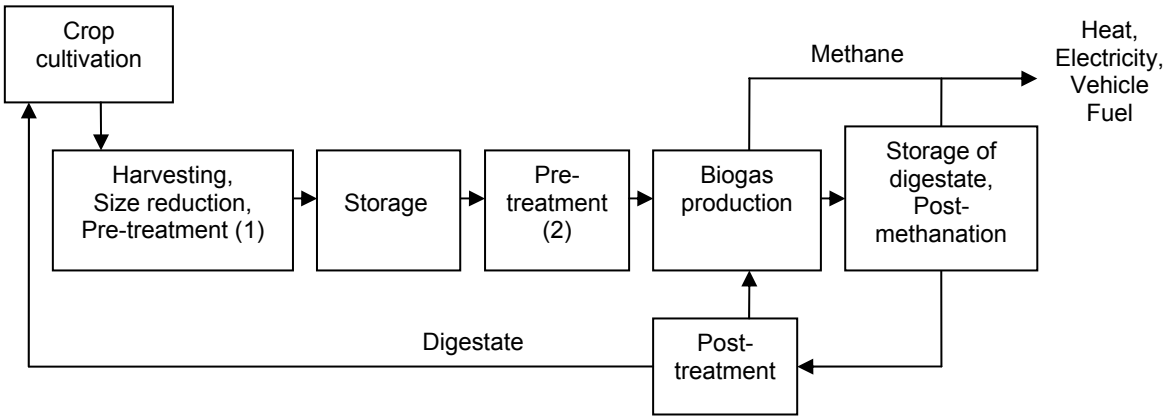

FIGURE 1 "Biogas from energy crops" –production chain (modified from Weiland 2003).

1.1 Selection of crops for methane production

The most important parameter in choosing crops for methane production is the net energy yield per hectare, which is defined mainly by biomass yield and convertibility of the biomass to methane, as well as cultivation inputs. Boreal energy crops should be easy to cultivate, harvest and store, tolerant to weeds, pests, diseases, drought and frost, have good winter hardiness and be able to grow on soil of poor quality with low nutrient input. Different crops have been screened for their methane potential on various occasions (reviewed by Gunaseelan 1997). Extensive screening has been performed with crops from different climatic areas, for example in Central Europe (Zauner & Kuntzel 1986, Zubr 1986, Weiland 2003), Florida USA (Shiralipour & Smith 1984, Chynoweth et al. 1993) and New Zealand (Badger et al. 1979, Stewart et al. 1984) (Table 1). However, very little is known about the methane potentials of crops suitable for biomass production in boreal areas. Furthermore, many studies have only considered the convertibility of the biomass to methane, and methane potentials have rarely been evaluated with regard to the biomass yields of crops and the corresponding methane and energy potentials per hectare (Table 1).

Many conventional forage crops are easy to cultivate and produce large amounts of biomass. Moreover, they have the advantage of being familiar to farmers and suitable for harvesting and storing with the existing methods and machinery. Furthermore, being bred for animal feed these crops are often characterised by good digestibility. Perennial herbaceous grasses (e.g. timothy *Phleum pratense* and reed canary grass *Phalaris arundinacea)* are among the most efficient producers of herbaceous biomass in boreal conditions, and many are commonly cultivated as forage in northern countries (Cherney et al. 1980, Lewandowski et al. 2003). Leguminous crops (e.g. red clover *Trifolium pratense,* vetch *Vicia sativa* and lupine *Lupinus polyphyllus)* form root nodules with the ability to bind nitrogen from the atmosphere. Thus, they require little fertilisation and contribute to efficient turnover of nitrogen in agriculture (Hyytiäinen et al. 1999). In addition to conventional forage crops, several less conventional agricultural species could have potential as energy crops. Examples of crops that are relatively easy to cultivate and produce plenty of biomass are marrow kale *Brassica olearacea* spp. *acephala*, Jerusalem artichoke *Helianthus tuberosus* and rhubarb *Rheum rhabarbarum*. Other species often identified as weeds (e.g. nettle *Urtica dioica* L. and giant knotweed *Reynoutria sachalinensis)* are an interesting alternative as energy crops due to their efficiency in photosynthesis, high competitiveness, ability to grow on soil of poor quality, wide distribution and fewer pests and diseases than with conventional forage crops (Callaghan et al. 1985a, Gilreath 1986). Furthermore, native weeds are invasive and resilient in nature, making them well suited for repeated harvesting (Callaghan et al. 1985a). A number of crop residues, such as sugar beet tops and straw, generated in large amounts in agriculture could also be utilised as a substrate in biogas production. Harvesting crop residues for energy use has the advantage that the direct costs of production of these materials are often low, and collecting them from the fields promotes nitrogen recycling and reduces eutrophication due to nitrogen leaching (Börjesson & Berglund 2003).

1.2 Effect of harvest time on the methane potential of energy crops

Methane production of a specific crop is affected by the chemical composition of the plant which changes as the plant matures (Cherney et al. 1986, Gunaseelan 1997), and timing and frequency of harvest are thus critical in order to optimise the biomass yield and feedstock quality (Callaghan et al. 1985b). Clover harvested at the vegetative stage was reported to yield up to 50% more methane per VS than at the flowering stage (Kaparaju et al. 2002), but in another study clover harvested at the vegetative stage had 32% lower methane potential per VS than at the flowering stage (Pouech et al. 1998) (Table 2). Harvest time had little effect on methane production of wheat, but rye grass harvested at flowering stage produced 50% more methane per VS than when harvested at vegetative stage (Pouech et al. 1998) (Table 2). With napier grass, the methane potentials per VS increased but the rate constants for methane

production decreased with harvest frequency (Chynoweth et al. 1993). With whole crop cereals, the methane potentials per VS of barley and rye increased by 11–14% when harvest was postponed to the milky stage, whereas with triticale harvest at the flowering stage was optimal (Heiermann et al. 2002) (Table 2). Thus, not much is known about the effect of harvest time on methane potentials of energy crops, and the results obtained thus far have been partly inconsistent (Table 2).

TABLE 1 Examples of the methane and gross energy potentials of energy crops and crop residues as reported in the literature.

Substrate	Methane potential				Gross energy potential	Ref.
	(m^3 CH_4 kg^{-1} VS_{added})	(m^3 CH_4 kg^{-1} TS_{added})	(m^3 CH_4 t^{-1} ww)	(m^3 CH_4 ha^{-1} a^{-1})	(MWh ha^{-1} a^{-1})	
Forage beet	0.46	n.r.	n.r.	5 800 [a]	56 [ac]	1
"	0.36	0.32 [c]	55 [c]	3 240 [b]	34 [b]	2
Alfalfa	0.41	n.r.	n.r.	3 965 [a]	38 [ac]	1
"	0.32	0.28 [c]	56 [c]	2 304 [b]	24 [b]	2
Potato	0.28	n.r.	n.r.	2 280 [a]	22 [ac]	1
Maize	0.41	n.r.	n.r.	5 780 [a]	56 [ac]	1
Wheat	0.39	n.r.	n.r.	2 960 [a]	28 [ac]	1
Barley	0.36	n.r.	n.r.	2 030 [a]	20 [ac]	1
Rape	0.34	n.r.	n.r.	1 190 [a]	12 [ac]	1
Grass	0.41	n.r.	n.r.	4 060 [a]	39 [ac]	1
"	0.27	0.24 [c]	46 [c]	1 908 [b]	20 [b]	2
"	0.27–0.35	0.25–0.32	64–83	n.r.	n.r.	3
Clover	0.35	n.r.	n.r.	2 530 [a]	25 [ac]	1
"	0.14–0.21	0.12–0.19	24–36	n.r.	n.r.	3
Marrow	0.26	n.r.	n.r.	1 680 [a]	16 [ac]	1
kale	0.32	0.28 [c]	42 [c]	2 304 [b]	24 [b]	2
Jerusalem artichoke	0.27	0.24 [c]	49 [c]	2 862 [b]	30 [b]	2
Sugar beet	0.23	0.19 [c]	n.r.	n.r.	n.r.	4
tops	0.36–0.38	0.29–0.31 [c]	36–38 [c]	n.r.	n.r.	5
Straw	0.25–0.26	0.23–0.24	139–145	n.r.	n.r.	3
"	0.30 [c]	0.25 [c]	n.r.	n.r.	n.r.	6

[a] in Germany, [b] in Sweden, [c] Values calculated from the data reported, VS = volatile solids, TS = total solids, t ww = tons of wet weight, a = year, MWh = megawatt-hour, n.r. = not reported. 1: Weiland 2003, 2: Brolin et al. 1988, 3: Kaparaju et al. 2002, 4: Gunaseelan 2004, 5: Zubr 1986, 6: Badger et al. 1979.

TABLE 2 Examples on the effect of harvest time on methane potentials of energy crops as reported in the literature.

Substrate	Stage of growth at harvest	Methane potential (m^3 CH_4 kg^{-1} VS_{added})	(m^3 CH_4 kg^{-1} TS_{added})	(m^3 CH_4 t^{-1} ww)	Ref.
Clover	vegetative	0.14–0.21	0.13–0.19	24–36	1
	flowering	0.14	0.12	17	1
Clover	vegetative	0.38	n.r.	n.r.	2
	budding	0.55	n.r.	n.r.	2
	flowering	0.56	n.r.	n.r.	2
Rye grass	vegetative	0.42	n.r.	n.r.	2
	earing	0.62	n.r.	n.r.	2
	flowering	0.63	n.r.	n.r.	2
Wheat	flowering	0.42	n.r.	n.r.	2
	milky	0.39	n.r.	n.r.	2
	pasty	0.38	n.r.	n.r.	2
Barley	flowering	0.44	0.40 [a]	74 [a]	2
	milky	0.50	0.47 [a]	129 [a]	2
	pasty	0.35	0.33 [a]	155 [a]	2
Rye	flowering	0.37	0.34 [a]	85 [a]	3
	milky	0.41	0.38 [a]	112 [a]	3
	pasty	0.28	0.27 [a]	164 [a]	3
Triticale	flowering	0.53	0.50 [a]	177 [a]	3
	milky	0.46	0.44 [a]	148 [a]	3
	pasty	0.34	0.33 [a]	215 [a]	3

[a] Values calculated from the data reported, n.r. = not reported. 1: Kaparaju et al. 2002, 2: Pouech et al. 1998, 3 : Heiermann et al. 2002.

1.3 Storage of crops intended for methane production

The production of biogas throughout the year, or at desired periods, necessitates the storage of the substrates used. As opposed to many other forms of renewable energy, such as wind or solar energy, energy crops and crop residues can relatively easily be stored and converted to energy at the desired point in time. Moreover, in northern conditions the growing season is relatively short, and the seasonal availability of plant material promotes the need to store the substrate. Plant material contains high levels of non-structural carbohydrates, which can be degraded during suboptimal storage conditions. Owing to high losses of organic matter and vulnerability to weather conditions, drying is not a favoured method when crops are used for biogas production; instead methods based on ensiling are often preferred (Egg et al. 1993).

Ensiling is a biochemical process that has been used to preserve forages for animal feed for centuries. During a typical ensiling process, the soluble carbohydrates contained in plant matter undergo lactic acid fermentation, leading to a drop in pH and to inhibition of the growth of detrimental micro-organisms. Lactic acid fermentation can be controlled by either preventing the growth of all micro-organisms by the addition of acids or by stimulating the growth of lactic acid bacteria (LAB) by adding, *e.g.,* a bacterial inoculum or enzymes. Fibrolytic enzymes degrade the plant cell walls and release intracellular soluble carbohydrates for lactic acid fermentation (McDonald et al. 1991). Ensiling thus produces intermediates for methanogenic fermentation,

and the structural polysaccharides contained in plant material, which are quite resistant to anaerobic degradation, can be partially degraded during storage. Storage can thus be considered a pre-treatment which simultaneously has potential to promote methane production from plant matter (Zubr 1986, Egg et al. 1993, Madhukara et al. 1993). While many authors have reported various crops stored as silage as having equal or higher methane potentials to those of fresh crops, the silages have been prepared without additives in the experiments reported thus far (Stewart et al. 1984, Gunnarson et al. 1985, Zubr 1986, Woodard et al. 1991, Chynoweth et al. 1993, Madhukara et al. 1997, Pouech et al. 1998, Heiermann et al. 2002, Rani & Nand 2004) (Table 3). Furthermore, in most of the earlier studies, the effect of lost organic matter during storage has not been considered in estimating the methane potentials of stored crops. To our knowledge, the effect of silage additives on the methane potential of crops has been previously reported only in the ensiling of whole crop maize, where the addition of LAB inoculant or amylase did not show improvement in methane potentials after 119 days of storage, calculated with storage losses taken into account and compared with crop stored without additives (Neureiter et al. 2005).

1.4 Pre-treatment of crops intended for methane production

Crop biomass mainly consists of cellulose, hemicellulose and lignin, i.e. lignocellulose. In addition to these compounds, crop biomass can contain e.g. non-structural carbohydrates (such as glucose, fructose, sucrose and fructans), proteins, lipids, extractives and pectins (McDonald et al. 1991). Lignin is poorly degraded in anaerobic conditions, and the rate and extent of lignocellulose utilisation is severely limited due to the intense cross-linking of cellulose with hemicellulose and lignin. Moreover, the crystalline structure of cellulose prevents penetration by micro-organisms or extracellular enzymes (Fan et al. 1981). As the rate-limiting step in anaerobic digestion of solid materials such as energy crops and crop residues is hydrolysis of complex polymeric substances (Noike et al. 1985, Mata-Alvarez et al. 2000), such as lignocellulose, one way of improving the methane production from anaerobic digestion of lignocellulosics is pre-treatment of the substrate in order to break the polymer chains to more easily accessible soluble compounds. An ideal pre-treatment would increase surface area and reduce lignin content and crystallinity of cellulose (Fan et al. 1981). Pre-treatments can be carried out either physically, chemically or biologically, or as combinations of these. Pre-treatments have been quite intensively studied for facilitating the enzymatic hydrolysis and consequent ethanol production from lignocellulosic substrates (Sun & Cheng 2002), but there is less information available on the effects of pre-treating crop biomass for methane production.

TABLE 3 Examples on the effect of storage (ensiling without additives) on methane potentials of energy crops and crop residues as reported in the literature.

Crop	Duration of storage (months)	Methane potential (m^3 CH_4 kg^{-1} VS_{added})		Change [a] (%)	Ref.
		Fresh	Silage		
Barley, flowering	3	0.438	0.462	5	1
Barley, milky	3	0.503	0.658	31	1
Rye, flowering	3	0.370	0.476	29	1
Rye, milky	3	0.410	0.492	20	1
Triticale, flowering	3	0.534	0.555	4	1
Triticale, milky	3	0.461	0.509	10	1
Energy cane	n.r.	0.245	0.265	8	2
Napier grass	n.r.	0.260	0.310	19	2
Napier grass	n.r.	0.248	0.264	6	2
Napier grass	n.r.	0.257	0.264	3	2
Pearl millet	n.r.	0.257	0.304	18	2
Pearl millet	n.r.	0.278	0.329	18	2
Sugar beet tops	6–12	0.360	0.381	6	3
Mustard	6–12	0.300	0.326	9	3
Rape	6–12	0.334	0.330	-1	3
Cauliflower	6–12	0.352	0.341	-3	3
White cabbage	6–12	0.382	0.343	-10	3
Rhubarb	6–12	0.316	0.345	9	3
Comfrey	6–12	0.334	0.323	-3	3
Jerusalem artichoke	6–12	0.309	0.301	-3	3
Jerusalem artichoke	n.r.	0.250 [b]	0.265 [b]	6	4
Jerusalem artichoke	n.r.	0.307 [b]	0.281 [b]	-8	4
Maize	4	0.383	0.480	25	5
Pineapple peel	6	0.28 [b]	0.44 [b]	57	6
Rye grass	n.r.	0.390	0.409	5	7
Green pea shells	6	0.35 [c]	0.38 [c]	9	8
Kale	1–3	0.231 [d]	0.304 [d]	32	9
Kale	4	0.231 [d]	0.260 [d]	13	9

[a] Change in methane potential during storage, [b] Values calculated from the data reported, [c] Expressed as m^3 biogas kg^{-1} VS_{added}, [d] Expressed as m^3 CH_4 kg^{-1} $TS_{added.}$, n.r. = not reported. 1: Heiermann et al. 2002, 2: Chynoweth et al. 1993, 3: Zubr 1986, 4: Gunnarson et al. 1985, 5: Neureiter et al. 2005, 6: Rani & Nand 2004, 7: Pouech et al. 1998, 8: Madhukara et al. 1997, 9: Stewart et al. 1984.

The most important physical pre-treatment of crop biomass is particle size reduction, leading to increase in available surface area and release of intracellular components (Palmowski & Müller 1999). With most substrates, there is a threshold value under which further reduction in particle size becomes uneconomical (Chynoweth et al. 1993). The results reported on the effect of particle size reduction on methane potential of lignocellulosic materials have been partly inconsistent (Table 4). For example, in batch assays with Bermuda grass, wheat straw and paddy straw, methane yields increased with decrease in particle size, but the difference between the smallest particle sizes tested (0.088 and 0.40 mm) was small (Sharma et al. 1988). Chynoweth et al. (1993) concluded that particle sizes in the millimetre to centimetre range would not significantly expose more surface area, and would exhibit similar methane production with sorghum and energy cane. According to Kaparaju et al. (2002), no difference in methane production from oat was observed between the tested particle sizes of 5, 10 and 20 mm, whereas the 10 mm particle size was found optimal for grass and least optimal for clover. Moreover, most of these tests

have been performed in laboratory methane potential assays, on the basis of which it is difficult to determine the practical importance of the results for full scale operation.

Other physical pre-treatments offering potential for improving methane yields from lignocellulosic materials are, for example, steam explosion, thermal hydrolysis, wet oxidation, pre-incubation in water, and treatment with ultrasound or radiation (Hashimoto 1986, Sharma et al. 1989, Sun & Cheng 2002, Fox & Noike 2004). Chemical pre-treatments include treatments with acids, alkalis, solvents or oxidants (Sun & Cheng 2002). Alkaline treatment is known to break the bonds between hemicellulose and lignin and to swell the fibres and increase the pore size, therefore facilitating hydrolysis (Datta 1981, Baccay & Hashimoto 1984, Pavlostathis & Gossett 1985). Pre-treatment with acids can be used to hydrolyse components of lignocellulose (Datta 1981, Sun & Cheng 2002). In biological pre-treatments either microbes and/or microbial enzymes are used for partial degradation of lignocellulose. These methods bear the advantage that they are usually simple and do not require major capital investments. However, the reported increases in biogas yields have been relatively low so far (Lissens et al. 2003). White-rot fungi are the only known living organisms capable of complete lignin degradation, and their application has been suggested for partial delignification to increase digestibility (Müller & Trösch 1986, Ghosh & Bhattacharyya 1999). Partial composting has also been suggested as a pre-treatment to anaerobic digestion (Jagadeesh et al. 1990, Kalia & Kanwar 1990). Composting is a bio-oxidative process involving the mineralisation and partial humification of organic matter (Delgenès et al. 2003) and lignin degradation has been reported in the thermophilic stage of composting (Tuomela et al. 2000). When designing appropriate pre-treatment methods for anaerobic digestion of crop biomass, the costs, practicability and environmental impacts of pre-treatments, as well as the losses of organic matter and energy content of substrates during pre-treatments, need to be weighed against the overall benefits of pre-treating the biomass (Datta 1981, Lehtomäki et al. 2004, Sun & Cheng 2002).

We have previously screened various pre-treatment methods for improving the methane potential of grass (Lehtomäki et al. 2004, Table 5). Highest increases of 17% in methane potential of grass were observed after 72 h (20 °C) treatment with alkalis (2% NaOH, and 3% $Ca(OH)_2$ + 4% Na_2CO_3) and 24 h (35 °C) treatment with enzymes (cellulases and hemicellulases). Pre-incubation in water (24 h at 35 °C) and autoclaving (30 min, 121 °C, 1 bar) also showed potential to increase the methane yields from grass by 13%. The white rot fungi treatment (21 d at 21°C) and short-term composting (7 d) resulted in high losses of organic matter due to biological activity, as a result of which the increase in methane potential was low or even negative (Lehtomäki et al. 2004, Table 5).

TABLE 4 Examples on the effect of particle size reduction on the methane potentials of lignocellulosic biomass as reported in the literature.

Substrate	Particle size (mm)	Methane potential (m^3 CH_4 kg^{-1} VS_{added})	Reference
Bermuda grass	0.088	0.226	Sharma et al. 1988
"	0.4	0.228	"
"	1.0	0.214	"
"	6.0	0.205	"
"	30.0	0.137	"
Wheat straw	0.088	0.249	"
"	0.4	0.248	"
"	1.0	0.241	"
"	6.0	0.227	"
"	30x5	0.162	"
Paddy straw	0.088	0.365	"
"	0.4	0.367	"
"	1.0	0.358	"
"	6.0	0.347	"
"	30x5	0.241	"
Sisal fibre	2	0.216	Mshandete et al. 2006
"	5	0.205	"
"	10	0.203	"
"	30	0.202	"
"	50	0.192	"
"	70	0.190	"
"	100	0.178	"
Wheat straw	0.5	0.327	Badger et al. 1979
"	20	0.255	"
Energy cane	ball milled	0.320	Chynoweth et al. 1993
"	0.8	0.240	"
"	8.0	0.290	"
Sorghum	1.6	0.420	"
"	8.0	0.410	"
Water hyacinth	1.6	0.16	Moorhead & Norstedt 1993
"	6.4	0.18	"
"	12.7	0.14	"
Clover	5	0.20	Kaparaju et al. 2002
"	10	0.14	"
"	20	0.21	"
Grass	5	0.32	"
"	10	0.35	"
"	20	0.27	"
Oat	5	0.26	"
"	10	0.25	"
"	20	0.25	"

TABLE 5 The effect of pre-treatments on the methane potential of grass according to Lehtomäki et al. (2004).

Pre-treatment	Methane potential (m^3 CH_4 kg^{-1} VS_{added})		Change (%)
	Before pre-treatment	After pre-treatment	
NaOH 2% 24 h 20 °C	0.23	0.25	9
NaOH 2% 72 h 20 °C	0.23	0.27	17
3% $Ca(OH)_2$ + 4% Na_2CO_3 24 h 20 °C	0.23	0.24	4
3% $Ca(OH)_2$ + 4% Na_2CO_3 72 h 20 °C	0.23	0.27	17
Autoclaving	0.23	0.26	13
Pre-incubation in water 24 h 35 °C	0.23	0.26	13
Enzyme 24 h 35 °C	0.23	0.27	17
White rot fungi 21 d 20 °C	0.23	0.24	4
Composting 7 d	0.23	0.19	-17

1.5 Reactor technology for anaerobic digestion of crop biomass: CSTR and leach bed processes

Energy crops and crop residues can be digested either alone or in co-digestion with other materials, employing either wet or dry processes. In the agricultural sector one possible solution to processing crop biomass is co-digestion together with animal manures, the largest agricultural waste stream. In addition to the production of renewable energy, controlled anaerobic digestion of animal manures reduces emissions of greenhouse gases, nitrogen and odour from manure management, and intensifies the recycling of nutrients within agriculture (Amon et al. 2006, Clemens et al. 2006). Animal manures typically have low solids content (<10% TS), and thus, the anaerobic digestion technology applied in manure processing is mostly based on wet processes, mainly on the use of continuously stirred tank reactors (CSTRs).

Co-digestion of animal manures with various agro-industrial residues has been reported previously (Callaghan et al. 2002, Kaparaju & Rintala 2005), with particular interest being shown in the co-digestion of animal manures with straws (Hills 1980, Fischer et al. 1983, Hashimoto 1983, Somayaji & Khanna 1994) (Table 6). However, there is little published data on the co-digestion of animal manures with energy crops (Weiland & Hassan 2001, Kaparaju et al. 2002) (Table 6), although in Germany, for example, more than half of the approximately 3 000 farm biogas plants in operation by the end of 2005 were using energy crops, mostly maize, in co-digestion with manures and other materials (Weiland 2005). Hashimoto (1983) and Fischer et al. (1983) reported lower specific methane yields in co-digestion of manure with straw compared with digestion of manure alone, but in another co-digestion study with digesters fed with cow manure and varying proportions of wheat straw, the highest specific methane yields were observed with 40% of wheat straw of TS in the feedstock (Somayaji & Khanna 1994, Table 6).

In co-digestion of plant material and manures, manures provide buffering capacity and a wide range of nutrients, while the addition of plant material with high carbon content balances the carbon to nitrogen (C/N) ratio of the

feedstock, thereby decreasing the risk of ammonia inhibition (Hills & Roberts 1981, Hashimoto 1983). The positive synergy effects often observed in co-digestion, due to the balancing of several parameters in the co-substrate mixture, have offered potential for higher methane yields (Mata-Alvarez et al. 2000). However, in digestion of crop materials in wet processes floating of the crop materials along with crust or scum formation has been reported (Nordberg & Edström 1997).

The gas production per digester volume, i.e. volumetric gas production, can be increased by operating the digesters at a higher solids concentration. Batch high solids reactors, characterised by lower investment costs than those of continuously fed processes, but with comparable operational costs, are currently applied in the agricultural sector to a limited extent (Köttner 2002, Weiland 2003). In these systems, digesters are filled with fresh substrate, with or without addition of inoculum, and allowed to go through all the degradation steps sequentially. Batch reactors are often leach bed processes where solids are hydrolysed by circulating leachate over a bed of organic matter. Recirculation of leachate stimulates the overall degradation owing to more efficient dispersion of inoculum, nutrients and degradation products (Chanakya et al. 1993, Lissens et al. 2001). Digestion of energy crops in one-stage leach bed processes has been seldom reported in the literature, but in batch leach bed processes digesting barley straw, reductions in VS of 45–60% and methane yields of 0.159–0.226 m^3 CH_4 kg^{-1} VS_{added} were obtained (Torres-Castillo et al. 1995) (Table 7), and in one-stage leach bed processes fed on a weekly basis with various lignocellulosic substrates (such as water hyacinth, straw, bagasse, cane trash etc.) and vegetable wastes, VS removals and biogas yields ranging from 37 to 86% and from 0.26 to 1.31 m^3 biogas kg^{-1} VS_{added}, respectively, were reported (Chanakya et al. 1993, 1997, Ramasamy & Abbasi 2000) (Table 7).

Batch leach bed processes can also be operated in conjunction with a second stage methanogenic reactor, with the leachate generated in the first stage pumped to the methanogenic reactor for further degradation (Ghosh 1984). Since the leachate has a low solids content, high-rate reactors such as upflow anaerobic sludge blanket reactors (UASBs) or anaerobic filters can be used in the second stage, and a high solid retention time is achieved in these reactors through the formation of granules or attachment of biomass to carriers (Henze & Harramoes 1983, Lettinga 1995). Anaerobic digestion of various agro-industrial wastes (Martinez-Viturtia et al. 1989, 1995, Mata-Alvarez et al. 1993, Zhang & Zhang 1999, Yu et al. 2002, Parawira et al. 2005, 2006) in processes of this kind has been reported in the literature (Table 8), but experiences from digestion of energy crops employing these processes are few. Methane yields and VS removals of 0.44 m^3 CH_4 kg^{-1} VS_{added} and 89%, and 0.27 m^3 CH_4 kg^{-1} VS_{added} and 60% have been reported in anaerobic digestion of sugar beet and grass silage, respectively, in laboratory batch leach bed processes connected to anaerobic filters (Cirne et al. 2006) (Table 8).

TABLE 6 Examples of co-digestion of animal manures and plant biomass in CSTRs operated within the mesophilic temperature range as reported in the literature (footnote on the following page).

Feedstock (ratio on VS basis, unless otherwise stated)	Reactor volume (l)	T (°C)	Feed TS (%)	OLR (kg VS m^{-3} d^{-1})	HRT (time of operation) (d)	VS removal (%)	Spec. CH_4 yield (m^3 CH_4 kg^{-1} VS_{added})	CH_4 (%)	Ref.
Pig manure, corn stover (75:25)	30	39	8	3.8	16	46	0.210 [a]	67	1
Cow manure	0.3	35	7.3	n.r.	15 (65)	n.r.	0.350	57	2
Cow manure, wheat straw (50:50)	0.3	35	7.8	n.r.	15 (65)	n.r.	0.100	31	2
Cow manure, wheat straw (25:75)	0.3	35	7.6	n.r.	15 (65)	n.r.	0.070	16	2
Cow manure, wheat straw (10:90)	0.3	35	7.9	n.r.	15 (65)	n.r.	0.030	16	2
Cow manure	20	35	3	4	n.r.	n.r.	0.100 [a]	n.r.	3
Forage beet silage	20	35	11	4	n.r.	93	0.500 [a]	53	3
Cow manure, forage beet silage (83:17 [a])	20	35	7[a]	4	n.r.	n.r.	0.400 [a]	55	3
Cow manure	18	35	7.6	3.6	21 (120)	51	0.240	n.r.	4
Cow manure, fruit and vegetable waste (FVW) (80:20, ww basis)	18	35	n.r.	4.2	21 (28)	51	0.380	n.r.	4
Cow manure, FVW (70:30, ww basis)	18	35	n.r.	4.5	21 (28)	30	0.340	n.r.	4
Cow manure, FVW (60:40, ww basis)	18	35	n.r.	5.2	21 (28)	50	0.450	n.r.	4
Cow manure, FVW (50:50, ww basis)	18	35	n.r.	5.0	21 (28)	46	0.380	n.r.	4
Cow manure	120	35–	n.r.	n.r.	22 (~140)	40–50	0.220	55–58	5
Cow manure, energy crops (n.r.)	120	37	n.r.	n.r.	22 (~20)	40–50	0.210	55–58	5
Pig manure	3.5	35	n.r.	2	44 (10)	n.r.	0.130–0.150	61–63	6
Pig manure, potato waste (85:15)	3.5	35	n.r.	2	39 (58)	n.r.	0.210–0.240	60–63	6
Pig manure, potato waste (80:20)	3.5	35	n.r.	2	26 (41)	n.r.	0.300–0.330	60–62	6
Pig manure, potato waste (80:20)	3.5	35	n.r.	3	39 (28)	n.r.	0.280–0.300	58–63	6
Cow manure, barley straw (80:20, volume basis)	100	35	17	5.2	25 (126)	29	0.160 [a]	65	7
"	100	35	17	6.5	20 (105)	28	0.170 [a]	64	7
"	100	35	17	8.7	15 (77)	26	0.150 [a]	61	7
"	100	35	17	12.5	10 (70)	24	0.110 [a]	58	7
Pig manure	20 [b]	35	n.r.	3.5	15 (74)	n.r.	0.320 [a]	62	8
Pig manure, wheat straw (75:25)	20 [b]	35	n.r.	3.8	15 (74)	n.r.	0.240 [a]	60	8
Pig manure, wheat straw (50:50)	20 [b]	35	n.r.	3.8	15 (74)	n.r.	0.220 [a]	58	8

(Table continues)

TABLE 6 (continues)

Feedstock (ratio on VS basis, unless otherwise stated)	Reactor volume (l)	T (°C)	Feed TS (%)	OLR (kg VS m^{-3} d^{-1})	HRT (time of operation) (d)	VS removal (%)	Spec. CH_4 yield (m^3 CH_4 kg^{-1} VS_{added})	CH_4 (%)	Ref.
Cow manure	2 [c]	n.r.	10	n.r.	40 (40)	27	0.107 [d]	60	9
Cow manure, wheat straw (80:20, TS basis)	2 [c]	n.r.	10	n.r.	40 (40)	37	0.109 [d]	58	9
Cow manure, wheat straw (60:40, TS basis)	2 [c]	n.r.	10	n.r.	40 (40)	32	0.113 [d]	59	9
Cow manure, wheat straw (40:60, TS basis)	2 [c]	n.r.	10	n.r.	40 (40)	34	0.103 [d]	59	9
Cow manure, wheat straw (20:80, TS basis)	2 [c]	n.r.	10	n.r.	40 (40)	32	0.097 [d]	58	9
Wheat straw	2 [c]	n.r.	10	n.r.	40 (40)	33	0.087 [d]	59	9

[a] values calculated from the data reported, [b] daily fed, periodically mixed digester, [c] no mention of mixing, [d] m^3 CH_4 kg^{-1} TS_{added}, OLR = organic loading rate, HRT = hydraulic retention time, n.r. = not reported. References: 1: Fujita et al. 1980, 2: Hashimoto 1983, 3: Weiland & Hassan 2001, 4: Callaghan et al. 2002, 5: Kaparaju et al. 2002, 6: Kaparaju & Rintala 2005, 7: Hills 1980, 8: Fischer et al. 1983, 9: Somayaji & Khanna 1994.

TABLE 7 Examples of anaerobic digestion of plant biomass in one-stage leach bed processes, as reported in the literature.

Feedstock	Mode of feeding	Reactor volume (l)	T (°C)	Feedstock TS (% ww)	VS removal (%)	Spec. CH_4 yield (m^3 CH_4 kg^{-1} VS_{added})	Ref.
Barley straw	Batch	220	35	35–36 [a]	45–60	0.159–0.226	1
Water hyacinth	Weekly	2	21–27	9.4	n.r.	0.348 [b]	2
Paddy straw	Weekly	6	26	n.r.	56.5	0.48 [ab]	3
Bagasse	Weekly	6	26	n.r.	37.1	0.83 [ab]	3
Cane trash	Weekly	6	26	n.r.	49.8	0.26 [ab]	3
Synedrella	Weekly	6	26	n.r.	68.1	0.95 [ab]	3
Parthenium	Weekly	6	26	n.r.	78.1	0.71 [ab]	3
Paper mulberry	Weekly	6	26	n.r.	85.5	1.31 [ab]	3
Vegetable waste	Weekly	11	35	n.r.	n.r.	0.513–0.869 [b]	4

[a] values calculated from the data reported, [b] m^3 biogas kg^{-1} VS_{added}, n.r. = not reported. 1: Torres-Castillo et al. 1995, 2: Chanakya et al. 1993, 3: Chanakya et al. 1997, 4: Ramasamy & Abbasi 2000.

TABLE 8 Examples of anaerobic digestion of plant biomass in two-stage processes consisting of a leach bed reactor and a methanogenic reactor, as reported in the literature.

Feedstock	Mode of feeding in 1st stage	Type of reactor as 2nd stage	Reactor volume 1st stage / 2nd stage (l)	T (°C)	Feedstock TS (% ww)	VS removal (%)	Spec. CH_4 yield (m^3 CH^4 kg^{-1} VS_{added})	Ref.
Fruit and vegetable waste	Batch	UASB-MF	1.3 / 0.5	35	5	83	0.345	1
Fruit and vegetable waste	Batch	UASB-MF	1.3 / 0.5	35	5	82	0.355	1
Fruit and vegetable waste	Batch	UASB-MF	1.3 / 0.5	35	5	87	0.368	1
Fruit and vegetable waste	Batch	UASB-MF	1.3 / 0.5	35	5	90	0.383	1
Fruit and vegetable waste	Batch	UASB-MF	1.3 / 0.5	35	5	n.r.	0.503	1
Fruit and vegetable waste	Batch	UASB-MF	1.3 / 0.5	35	5.5	80	0.507	2
Fruit and vegetable waste	Daily	UASB-MF	1.3 / 0.5	35	6.4	72	0.405 [a]	3
Fruit and vegetable waste	Daily	UASB-MF	1.3 / 0.5	35	6.4	53	0.294 [a]	3
Fruit and vegetable waste	Daily	UASB-MF	1.3 / 0.5	35	6.4	38	0.187 [a]	3
Fruit and vegetable waste	Daily	UASB-MF	1.3 / 0.5	35	6.4	27	0.098 [a]	3
Potato waste	Batch	UASB	2.0 / 0.84	37	19	n.r.	0.39	4
Potato waste	Batch	MF	2.0 / 1.0	37	19	n.r.	0.39	4
Sugar beet leaves	Batch	MF	7.6 / 2.6	35–37	n.r.	n.r.	0.216 [a]	5
Unpeeled potatoes	Batch	MF	7.6 / 2.6	35–37	n.r.	n.r.	0.258 [a]	5
Peeled potatoes	Batch	MF	7.6 / 2.6	35–37	n.r.	n.r.	0.351 [a]	5
Sugar beet leaves, potatoes 1:2	Batch	MF	7.6 / 2.6	35–37	n.r.	n.r.	0.402 [a]	5
Sugar beet leaves, potatoes 1:3	Batch	MF	7.6 / 2.6	35–37	n.r.	n.r.	0.402 [a]	5
Grass waste	Batch	MF	8000 / 190	Ambient	92	67	0.165 [a]	6
Grass silage	Batch	MF	0.75 / 0.9	37	27	60	0.27	7
Sugar beet	Batch	MF	0.75 / 0.9	37	24	89	0.44	7
Rice straw	Batch	ASBR	4.0 / 4.0	35	92	44	0.19 [a]	8
Rice straw	Batch	ASBR	4.0 / 4.0	35	92	45	0.19 [a]	8
Rice straw	Batch	ASBR	4.0 / 4.0	35	92	48	0.21 [a]	8
Water hyacinth	Weekly	MF	2.0 / 0.5	n.r.	9.6	n.r.	0.181 [b]	9

UASB = upflow anaerobic sludge blanket reactor, MF = methanogenic filter, ASBR = anaerobic sequencing batch reactor. [a] values calculated from the data reported, [b] m^3 biogas kg^{-1} TS_{added}, n.r. = not reported. References: 1: Martinez-Viturtia et al. 1989, 2: Mata-Alvarez et al. 1993, 3: Martinez-Viturtia et al. 1995, 4: Parawira et al. 2005, 5: Parawira et al. 2006, 6: Yu et al. 2002, 7: Cirne et al. 2006, 8: Zhang & Zhang 1999, 9 : Chanakya et al. 1992.

1.6 Post-methanation and utilisation of digestates

In practice, not all of the methane potential in substrates can be extracted in anaerobic digestion within the reactor residence time, and if the digestates are stored in uncovered storage tanks without gas collection, part of this methane can be lost to the atmosphere through spontaneous degradation. Methane is a powerful greenhouse gas with 21 times higher global warming potential that that of carbon dioxide (IPCC 2001), and therefore, preventing methane emissions to the atmosphere is very desirable. Post-methanation of digestates in covered storage tanks offers the possibility of both minimising the potential methane emissions, as well as contributing to an increase in the obtainable methane yields (Kaparaju and Rintala 2003). The post-storage tanks can act as part of the required storage capacity on a farm; for example, in Finland storage capacity corresponding to the manure production of one year is required, as the spreading of manure on farmland is allowed only during the frost-free months (Finnish Government 1998). Depending on the feed materials and process conditions, typically 5–15% of the total biogas produced can be obtained from post-methanation of residues (Weiland 2003), and for example, the addition of potato starch as a co-digestate in cattle slurry digesters was reported to result in up to 30% higher post-methanation potential in the digestate (Clemens et al. 2006).

Production of inorganic fertilisers, especially the capture of nitrogen from atmosphere, is a very energy-intensive process. For example, in cultivation of grass the production and application of inorganic fertilizers accounts for 35% of total energy input (Börjesson 2004). In order to maintain a positive energy balance in farm-scale biogas production, the use of inorganic fertilisers in cultivation of energy crops should be minimised. The residues from anaerobic digestion contain mineralised nitrogen, which is readily available for growing plants, as well as residual carbon, phosphorous and trace nutrients, and they can thus be returned to the cultivation soil as a fertiliser and a soil-improvement medium (Demuynck 1984, Hons et al. 1993, Karpenstain-Machan et al. 2001). Additionally, anaerobically treated materials are often more viscous and less odorous than the original feestocks, making them easier to handle and spread, and anaerobic treatment is known to inactivate weed seeds, plant pathogens and pests, and decrease the amount of phytotoxic compounds e.g. in manure (Demuynck 1984, Engeli et al. 1993, Hons et al. 1993, Gunaseelan 1998).

2 OBJECTIVES

The main purpose of this thesis was to evaluate the feasibility of methane production from energy crops and crop residues through anaerobic digestion in boreal conditions by focusing on selected aspects of the "biogas from energy crops"-production chain (Fig. 1). The subobjectives were:

- to determine the suitability of various potential boreal energy crops and crop residues for methane production, taking into account the biomass yields per hectare (I),
- to determine the effect of harvest time on the methane potential of different crops (I),
- to determine the optimal methods for storing the methane potential in grass and sugar beet tops (II),
- to asses the viability of the co-digestion of energy crops and crop residues with cow manure, and to determine the results of treating increasing proportions of crop materials in co-digestion with manure along with the post-methanation potentials of the digestates (III),
- to evaluate the suitability of batch leach bed reactors for methane production from energy crops by comparing the operation of a one-stage process consisting of a batch leach bed reactor and a two-stage process consisting of batch leach bed reactors in connection with an UASB (IV) or a MF (V),
- to evaluate the suitability of the residues from anaerobic digestion of energy crops in leach bed – MF processes as fertilisers by determining the fate of nitrogen and heavy metals in this process (V).

3 MATERIALS AND METHODS

The materials and methods are described in more detail in the original articles (I–V).

3.1 Origin of materials

Potential energy crops and crop residues were screened for their suitability in methane production (I), and the samples used in these screening experiments were obtained from local farmers and companies, natural stands or private gardens nearby the institutes during summer 2002. Each potential energy crop was harvested at two different maturity stages, generally corresponding to the vegetative stage and flowering stage, and referred to as 1st and 2nd harvest, respectively (Table 9). For the purpose of presenting the results, the crops used in the screening tests (I) were grouped as grasses (timothy-clover grass, reed canary grass, lawn), legumes (red clover, vetch-oat, lupine), leafy crops (Jerusalem artichoke, giant knotweed, nettle, rhubarb, marrow kale, tops of sugar beet) and straws (straw of oats, straw of rapeseed). The substrates used in the storage experiments (II) were grass (seed mixture: 75% timothy *Phleum pratense*, 25% meadow fescue *Festuca pratensis*) harvested at the early flowering stage after 24 h of pre-wilting, and tops of sugar beet *Beta vulgaris* (Table 10). In laboratory reactor experiments (III and IV), grass silage obtained from a local farm in central Finland (Kalmari farm, Laukaa) was used (Table 10). It was prepared at the farm from grass (75% timothy *Phleum pratense*, 25% meadow fescue *Festuca pratensis*) harvested at early flowering stage, which was chopped with an agricultural precision chopper after 24 h of pre-wilting and ensiled in a bunker silo with the addition of a commercial silage additive (LAB inoculant AIV Bioprofit, Kemira Growhow Ltd.). In the co-digestion experiment (III), straw of oat *Avena sativa* from a farm in central Finland (Kalmari farm, Laukaa) and tops of sugar beet *Beta vulgaris* from a farm in southern Finland (Koskela farm, Kaasmarkku) were also used as substrates (Table 10).

In the screening experiments (I), all harvested plant materials were taken to the laboratory and chopped to approximately 1 cm particle size either with scissors or a stainless steel knife. Grass used in storage and laboratory reactor experiments (II–IV) was chopped at harvest with an agricultural precision chopper, whereas sugar beet tops (II, III) and straw (III) were chopped with a garden chopper (Wolf Garten SD 180E) to a particle size of approximately 3 cm. Fresh materials were used in screening and storage experiments (I–II), whereas in laboratory reactor experiments (III–IV), the crop samples were frozen and stored at -20 °C. Before analysis and feeding to the reactors, the samples were allowed to thaw overnight at 4 °C.

The substrates used in pilot experiments (V) were energy willow *Salix viminalis* (run A), sugar beet *Beta vulgaris* (run B) and grass silage (run C) (Table 10). The shoots of willow were harvested and chopped with an agricultural precision chopper at the end of first growing season, and this fresh material was directly loaded to the digesters. Whole sugar beet, *i.e.* both the beets and tops were used as substrate in run B, and before loading to the reactors, the beet tops stored as silage where mixed with the stored beets in ratio 1:3 on ww basis and chopped in an agricultural mixer. Grass silage was prepared from a mixture of English ryegrass *Lolium perenne* (50%) and white clover *Trifolium repens* (50%) by chopping at harvest with an agricultural precision chopper and storing in bales. Before being loaded in the reactors the grass silage was mixed in an agricultural mixer.

The cow manure (III) was obtained from a dairy farm in central Finland (Kalmari farm, Laukaa) (Table 10).

The inoculum used to inoculate the methane potential assays (I–IV), post-methanation assays (III–IV) and reactor experiments (III–IV) was obtained from a mesophilic farm digester (Laukaa, Finland) treating cow manure and industrial confectionary by-products as substrate, whereas the inoculum used in methane potential assays with the substrates used in pilot experiments (V) was obtained from a mesophilic completely mixed low solids reactor treating crop residues as substrate (Table 11). The UASB (IV) was inoculated with granular sludge obtained from a full-scale internal circulation reactor treating wastewater from sugar and vegetable processing (Säkylä, Finland).

TABLE 9 Dates of harvest, maturity stages of energy crops at harvest, and characteristics of the substrates used in the screening experiment (I).

Substrate	Har-vest	Date of harvest	Stage of growth at harvest	TS (%ww)	VS (%ww)	VS / TS	N_{tot} (%TS)	C_{tot} (%TS)	C/N	$Lignin_{tot}$ (%TS)
ENERGY CROPS:										
Timothy-clover grass [a]	1	6 JUN	vegetative	24.8	23.0	0.93	1.8	46.2	26	15.5
	2	19 JUN	silage stage	20.2	18.8	0.93	3.4	46.8	14	19.3
Reed canary grass	1	26 JUN	early flowering	29.5	28.4	0.96	1.7	48.1	28	20.4
	2	8 AUG	late flowering	39.9	38.7	0.97	1.9	47.8	25	22.4
Red clover	1	27 JUN	vegetative	15.3	13.8	0.90	5.2	47.6	9	20.9
	2	12 AUG	flowering	26.2	24.1	0.92	3.2	47.5	15	21.6
Vetch-oat mixture [b]	1	17 JUL	vegetative	15.5	13.9	0.90	3.2	44.9	14	18.5
	2	12 AUG	flowering	25.6	24.1	0.94	2.6	46.6	18	18.6
Lupine	1	4 JUN	vegetative	12.4	11.2	0.90	3.5	47.4	13	19.0
	2	18 JUN	flowering	14.3	13.3	0.93	3.9	47.9	12	17.1
Jerusalem artichoke	1	27 AUG	vegetative	27.8	25.5	0.92	0.5	43.6	79	18.5
	2	19 SEP	flowering	32.4	30.3	0.94	0.9	43.5	49	17.8
Giant knotweed	1	27 JUN	vegetative	20.7	19.1	0.92	2.1	47.0	23	26.8
	2	27 AUG	flowering	30.3	28.3	0.93	1.2	47.2	41	28.0
Nettle	1	4 JUN	vegetative	15.0	12.3	0.82	4.2	41.0	10	18.9
	2	18 JUN	flowering	16.6	14.3	0.86	4.2	44.3	11	19.0
Rhubarb	1	4 JUN	vegetative	9.4	8.1	0.86	3.2	43.8	14	16.2
	2	6 JUN	flowering	9.1	7.9	0.87	2.5	43.5	18	24.4
Marrow kale	1	28 AUG	early vegetative	13.0	11.8	0.91	3.0	44.3	15	12.0
	2	18 SEP	late vegetative	13.1	11.8	0.90	2.4	43.5	18	13.5
CROP RESIDUES:										
Tops of sugar beet		1 OCT		11.6	9.9	0.85	2.2	39.9	18	9.8
Straw of oats		26 AUG		89.6	81.2	0.91	0.5	44.4	95	21.1
Straw of rapeseed		11 SEP		90.0	82.5	0.92	1.6	44.5	28	20.4
Lawn		3 JUN		21.5	19.1	0.89	0.7	46.4	71	25.9

[a] Composition of seed mixture: 67.5% timothy *Phleum pratense*, 22.5% meadow fescue *Festuca pratensis*, 10.0% red clover *Trifolium pratense*,
[b] Composition of seed mixture: 50.0% common vetch *Vicia sativa*, 50.0% oat *Avena sativa*. N_{tot} = total nitrogen, C_{tot} = total carbon, $lignin_{tot}$ = total lignin.

TABLE 10 Characteristics of the substrates used in the storage and reactor experiments (II–V).

Substrate	TS (%ww)	VS (%ww)	pH	N_{tot} (mg g^{-1} TS)	NH_4-N (mg g^{-1} TS)	SCOD (mg g^{-1} TS)	Lignin (%TS)	Paper
Grass (fresh)	30.2	27.9	6.4	39.2	0.8	n.d.	n.d.	II
Grass (silage)	25.9	24.0	3.9	16.9	1.4	228	17	III, IV
Grass (silage)	31.8	27.9	n.d.	37.0	3.3	n.d.	5	V
Sugar beet tops	11.2	9.1	6.4	27.7	0.2	n.d.	n.d.	II
Sugar beet tops	10.3	8.3	6.0	18.1	0.6	263	n.d.	III
Sugar beet (whole)	20.2	17.9	n.d.	9.0	0.1	n.d.	2	V
Oat straw	63.5	57.6	6.6	10.9	0.4	103	n.d.	III
Willow	49.5	48.7	n.d.	9.0	0.0	n.d.	14	V
Cow manure	6.5	5.3	7.4	41.5	15.8	233	n.d.	III

NH_4-N= ammonium nitrogen, SCOD = soluble chemical oxygen demand. n.d. = not determined.

TABLE 11 Characteristics of inocula used in laboratory experiments (I–V) (average values ± standard deviations).

Paper	TS (%ww)	VS (%ww)	pH	N_{tot} (g l^{-1})	NH_4-N (g l^{-1})	SCOD (g l^{-1})
I	5.6 ±0.7	4.4 ± 0.6	7.7 ± 0.1	3.4 ± 0.1	1.9 ± 0.1	16.4 ± 1.2
II	5.8 ±0.7	4.4 ± 0.7	7.7 ± 0.2	3.4 ± 0.5	0.7 ± 0.3	3.9 ± 1.1
III, IV	6.6 ± 0.3	5.0 ± 0.2	7.7 ± 0.2	3.2 ± 0.2	1.1 ± 0.1	12.4 ± 1.5
V	3.4 ± 0.9	1.8 ± 0.5	7.5 ± 0.1	1.5 ± 0.2	0.5 ± 0.1	7.5 ± 0.3

3.2 Experimental set-up

3.2.1 Storage experiments (II)

The storage methods studied in laboratory storage experiments with grass and sugar beet tops were storage with formic acid (FA), with enzymes, with LAB inoculant and with a mixed culture from a farm biogas reactor. As a control, the plant materials were stored under the same conditions without additives. The storage additives were dosed per fresh weight (FW) and manually mixed with plant material. FA (80% volume/volume, v/v) was applied to plant material in a volume/weight (v/w) ratio of 0.5%. Solution (1% v/v) of enzymes (two xylanases (GC 320 and Multifect) and two cellulases (IndiAge MAX L and Primafast 200) originating from *Trichoderma reesei*, Genencor International Ltd, in a v/v ratio of 1) was applied to plant material in a ratio of 0.01% (v/w). A commercial LAB inoculant, AIV Bioprofit, containing 60% *Lactobacillus rhamsonus* and 40% *Propionibacterium freudenreichii* spp. *shermanii* (Kemira Growhow Ltd.) with a total count of 5.8×10^{11} colony-forming units (CFU) g^{-1} was diluted to 0.7 g l^{-1} in tap water and applied to plant material in a ratio of 0.5% (v/w). The mixed culture was a supernatant (separated from solids by centrifugation at 2 800 revolutions $minute^{-1}$ (rpm) for 10 min in a household spin dryer (775 SEC 156 Centrifuge, Thomas) equipped with a nylon-woven fabric bag of 100 µm pore size) of digested sludge from the same digester as the

inoculum (see 3.1). The supernatant was applied to plant material in a ratio of 25% (v/w).

Each storage experiment was conducted in the laboratory in duplicate. Plant material (400 g TS) mixed with storage additives was packed and compressed by hand in polyethylene bags. The bags were then tightly sealed and placed in 5 l laboratory silos equipped with air-tight lids and water locks to enable gas release. After closing, the silos were flushed with N_2 gas and immediately stored at their respective storage temperatures. The durations of the storage experiments were three months at 20 °C, and six months at 20 and 5 °C. The silos were weighed before and after storage to determine the changes in crop mass during storage.

3.2.2 Co-digestion experiments (III)

Co-digestion experiments were carried out in four parallel laboratory continuously stirred tank reactors (CSTRs) (referred to as R1-R4) constructed of glass, each with a total capacity of 5 l and a liquid volume of 4 l, stirred continuously with magnetic stirrers at 300 rpm and incubated at 35±1 °C (III). Digesters were inoculated on day 0 with 4 l of inoculum. Thereafter the digesters were fed with a syringe semi-continuously (once a day, 5 days a week). Prior to feeding, an equivalent volume of digester content was removed. The applied feedstock mixtures were prepared daily.

3.2.3 Laboratory leach bed reactors and UASB (IV)

The laboratory leach bed reactors were plastic column reactors (1000 ml) operated with continuous leachate recirculation at 35 (±1) °C (IV). In one-stage operation, leachate was collected at the bottom of the reactor in a liquid reservoir (R1) and recycled back to the top of the reactor. The pH of the recirculated leachate was adjusted to 7 automatically with 1 M NaOH (pH was allowed to vary between 6.9 and 7.1) (run 1, Fig. 2). In two-stage operation, the leachate from the leach bed reactor was collected in a leachate reservoir (R1), from which it was circulated to an UASB, a glass reactor with 1000 ml liquid volume. The UASB effluent was collected in another reservoir (R2), from which it was recirculated back to the top of the leach bed reactor (run 2 and 3, Fig. 2). The biogas produced was collected from the top of each reactor and liquid reservoir into aluminium gas bags. Before starting the experiments the UASB had been inoculated with granular sludge (see 3.1) and operated for two months with a synthetic substrate (distilled water with the following additives (concentration mg l^{-1}): molasses (5000), NH_4Cl (1000), KH_2PO_4 (165), KCl (165), $CaCl_2$ • 2 H_20 (50), $MgCl_2$ • 6 H_2O (100), yeast exctract (500), $NaHCO_3$ (3000); pH adjusted to 7.5) at an OLR of 5 kg COD m^{-3} d^{-1}.

In runs 1–3, the leach bed reactors were filled with 50 g VS (208 g ww) of grass silage and 750 ml of tap water was added to obtain an initial liquid/solid (L/S) ratio of 17. In one-stage operation (run 1), the substrate was mixed with 3.2 g VS (64 g ww) of inoculum before feeding to the reactor, and the recirculation rate of leachate was adjusted to 750 ml d^{-1} throughout the run. In the beginning of two-stage operation (runs 2 and 3), the leach bed reactors were

operated with internal recirculation, with the leachate collected in R1 returned on top of the leach bed at a flow rate corresponding to 750 ml d^{-1}, for 24 h, after which the leachate had reached a COD value higher than 10 g l^{-1}, and circulation of the leachate to the UASB was initiated. The loading rate to the UASB was then maintained at 5 kg COD m^{-3} d^{-1}, which determined the flow rate to both reactors. When the leachate COD decreased to below 2 g COD l^{-1}, circulation to the UASB was terminated and the leach bed reactors were operated as one-stage processes with internal recirculation, with a flow corresponding to 750 ml d^{-1}, until the end of the run.

The two-stage experiments were conducted without (run 2) and with (run 3) pH adjustment (Fig. 2). In the latter case, the pH of the effluent from the UASB was automatically adjusted to 6 with 1 M hydrochloric acid (HCl) (pH was allowed to vary between 5.9 and 6.1) before entering the leach bed reactor (Fig. 2). One-stage operation and two-stage operation without pH adjustment was continued for 55 days, whereas the two-stage operation with pH adjustment was continued for 31 days.

In run 4, six parallel leach bed reactors (LB1–6) and one UASB were operated (Fig. 2). The leach bed reactors and UASB were identical to those in runs 2 and 3, and they were operated in a similar fashion, except that the leach bed reactors were installed in parallel, so that leachate from all six reactors was collected in a common reservoir (R1) and circulated from there to the common UASB. The effluent from the UASB was collected in a reservoir (R2) and circulated back to the top of the leach bed reactors so that each reactor received the same liquid at the same flow rate. At start-up, the leach bed reactors were filled with 50 g VS (208 g ww) of grass silage without inoculum, after which 250 ml of tap water was added per reactor (1500 ml in total) (initial L/S ratio 8). The leach bed reactors were initially operated with internal recirculation, with the leachate collected in R1 returned on top of the leach bed reactors at a flow rate corresponding to 250 ml d^{-1} $reactor^{-1}$. Circulation to the UASB was then initiated on day 1 and terminated on day 17, after which the leach bed reactors were operated on internal recirculation, with a flow corresponding to 250 ml d^{-1} $reactor^{-1}$, until the end of the run.

The six parallel leach bed reactors in run 4 were terminated sequentially; LB1 was terminated on day one, LB2 on day 3, LB3 on day 6, LB4 on day 10, LB5 on day 17 and LB6 at the end of the run, on day 49. At each termination, 250 ml of leachate was removed from the system, and the reactor contents and leachate were sampled for analysis.

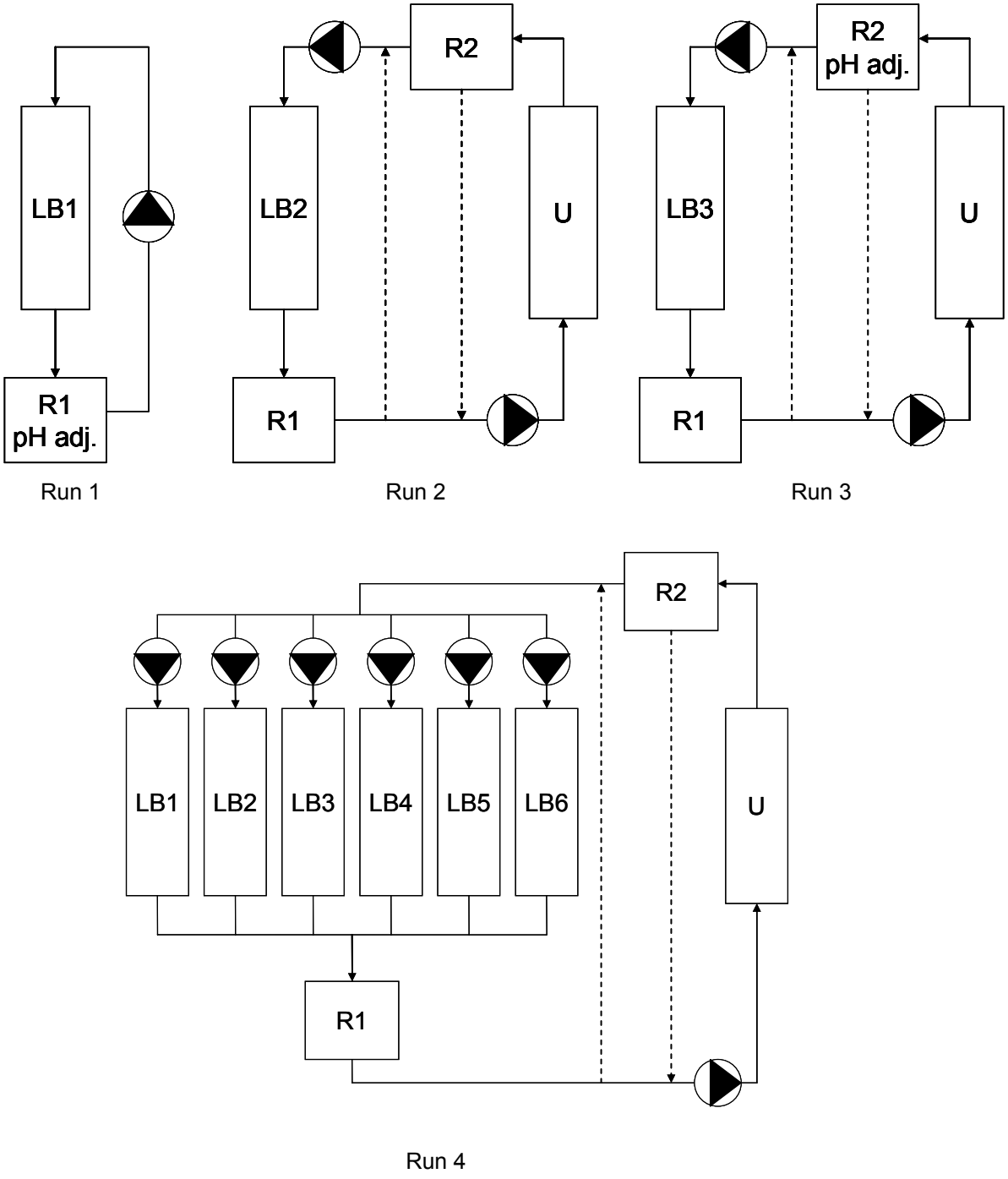

FIGURE 2 Reactor set-ups in the experiments with laboratory leach bed reactors and UASB (IV) (LB = leach bed reactor, U = UASB, R = liquid reservoir, pH adj. = pH adjustment). Dashed lines represent the flow of process liquid during internal recirculation.

3.2.4 Pilot leach bed reactors and methanogenic filters (V)

Two parallel pilot reactor set-ups (1 and 2) were used in V (Fig. 3). The leach bed reactor (1st stage, referred to as H1 in set-up 1 and H2 in set-up 2) of both set-ups consisted of a 10 m^3 (7.6 m^3 active volume) reactor equipped with leachate circulation. The 2nd stage of both reactor set-ups was a 2.6 m^3 methanogenic reactor (referred to as MF1 in set-up 1 and MF2 in set-up 2) built of stainless steel and equipped with leachate recirculation (Fig. 3). MF1 was a downflow methanogenic filter packed with pre-digested straw, whereas MF2 was an upflow methanogenic filter packed with plastic carriers (HUFO 120 m^2/m^3, Nordiska Plast AB). The effluents from the methanogenic filters (MFs) were recycled back to the respective leach bed reactor. In each set-up, the same pump was used for circulation over both stages, and the flow was switched between the 1st and the 2nd stage by pneumatic valves (Fig. 3). At each flow change, a set volume of 8 l was exchanged between the two reactors, and the number of circulation changes over a day determined the amount of liquid exchanged between the two reactors. All reactors were operated under mesophilic temperature conditions. At each start-up, the liquid in the 1st stages was replaced with fresh water, whereas the liquid in 2nd stages was reused and thus not replaced.

Substrate was added to the 1st stage in a removable cage in a batchwise manner, after which 2 m^3 of fresh water was added. Initially, the 1st stage was operated with internal recirculation of leachate, with the leachate collected from bottom sprayed on top of the bed. Circulation over the 2nd stage was initiated when the COD of the leachate reached a level of 10 g l^{-1} and was continued until pH in the 1st stage was higher than 7 and methane (CH_4) production had begun. Then the circulation over the 2nd stage was terminated and the leach bed reactor was operated as a one-stage process until the gas production became negligible and the runs were terminated.

The loading rates to the MFs varied between 3–19 kg COD m^{-3} d^{-1} (Table 12). If the pH in the effluent from the MFs decreased to below 7, the feeding was interrupted and the MFs were operated with internal circulation until the effluent pH was stabilised above 7. Durations, amounts of substrates added and temperature conditions in the different runs are presented in Table 12.

TABLE 12 The durations of runs, amounts of substrates added, OLRs to MFs and temperature conditions in pilot experiments (V) (standard deviations in parenthesis, where applicable).

Run	Substrate	Duration (d)	Amount of substrate added (kg ww) Reactor set-up		OLRs to MFs (kg COD m^{-3} d^{-1}) Reactor set-up		Temperature (°C) Reactor set-up			
			1	2	1	2	1		2	
							H1	MF1	H2	MF2
A	Willow	82	1300	1300	5–10, av. 6	5–10, av. 6	35.9 (0.1)	36.4 (1.9)	36.0 (0.1)	34.1 (4.7)
B	Sugar beet	55	1970	1842	3–10, av. 6	8–19, av. 11	34.9 (3.1)	36.6 (2.5)	36.3 (0.4)	36.7 (2.5)
C	Grass	50	1940	1940	5–20, av. 11	5–20, av. 11	36.2 (0.7)	36.6 (0.8)	36.1 (0.1)	36.7 (0.7)

av. = average value.

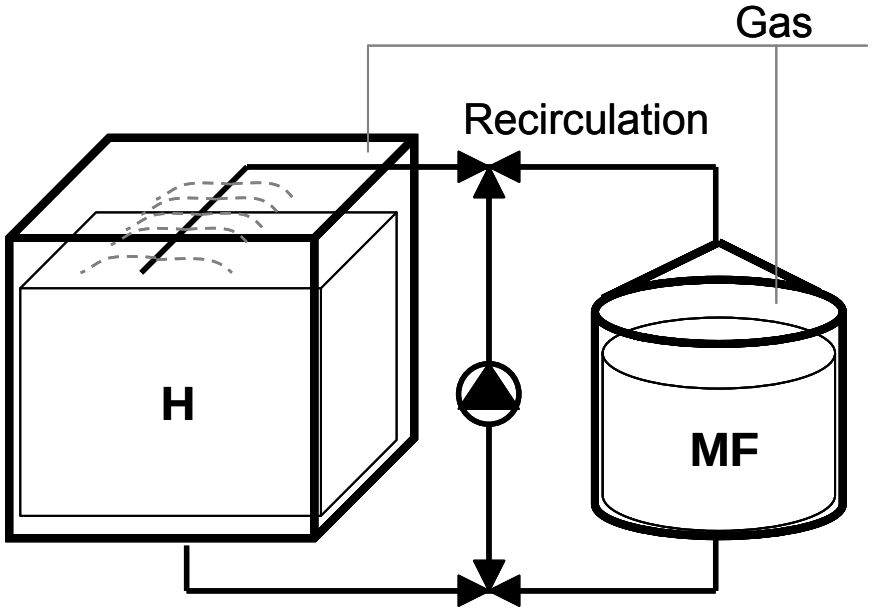

FIGURE 3 Schematic drawing of the reactor set-up 1 in pilot experiment (V). Set-up 2 is identical except for the reversed flow direction in MF2. Dashed lines represent the flow of process liquid.

3.2.5 Methane potential assays (I–V)

The methane potentials of all substrates were determined in batch experiments in duplicate or triplicate in either 2 l glass bottles (liquid volume 1.5 l) incubated statically at 35±1 °C (I–IV) or 500 ml bottles (liquid volume 300 ml) incubated on a shaking water bath (70 rpm) at 37±1°C (the samples of the crops used in pilot experiments, V). Inoculum (500 ml in I–IV, 250 ml in V) and substrate in a

$VS_{substrate}/VS_{inoculum}$ ratio of 1 were added into the bottles, distilled water was added to produce a liquid volume of 1.5 (I–IV) or 0.3 l (V), and sodium bicarbonate ($NaHCO_3$, 3 g l^{-1}) was added as buffer. The contents of the bottles were flushed with N_2/CO_2-gas for 5 minutes and the bottles were then sealed with butyl rubber stoppers. The gas produced was collected in aluminium gas bags. In I–IV, the bottles were mixed manually before each gas measurement. Assays with inoculum only were carried out to subtract the methane potential of the inoculum from that of the substrates. Methane potential assays with samples from storage experiments (II) were concluded after 42 days, whereas the assays with samples from crop screening and reactor experiments (I, III–V) were continued until methane production became negligible (< 5 ml CH_4 d^{-1}) after 60–140 d.

3.2.6 Post-methanation assays (III–IV)

The post-methanation potentials of the digestates were measured in batch experiments in triplicate 118 ml serum vials incubated either in 35 °C (digestates from leach bed processes, IV) or in 5, 20 and 35 °C (digestates from CSTRs, III). Either 40 g of digestate alone (III) or 1 g VS of both digestate and inoculum (IV) was added into the vials, after which the vials were sealed with butyl rubber stoppers and aluminium crimps, flushed with N_2/CO_2-gas for 5 minutes and incubated statically for 100 d at their respective incubation temperatures. Assays with inoculum only were carried out to subtract the methane potential of the inoculum from that of the substrates.

3.3 Analyses and calculations

Biogas samples were taken with a pressure lock syringe and their methane content was measured with gas chromatographs (GC) equipped with a flame-ionisation detector (I–II: Perkin Elmer Autosystem XL, Perkin Elmer Alumina column 30 m × 0.53 mm, carrier gas helium, oven 100 °C, injection port 250 °C, detector 225 °C; III: Perkin Elmer Clarus 500, Perkin Elmer Alumina column 30 m × 0.53 mm, carrier gas argon, oven 100 °C, injection port 250 °C, detector 225 °C) or with a thermal conductivity detector (IV: Perkin Elmer Clarus 500, Supelco Carboxen™ 1010 PLOT fused silica capillary column 30 m × 0.53 mm, carrier gas argon, oven 200 °C, injection port 225 °C, detector 230 °C; V: Agilent Technologies 6890 Network GC system, Haysep (N 80/100, 9 ft, 1/8) and Molesieve (5 A 60/80, 6 ft, 1/8) columns, carrier gas helium, oven 70 °C, injection port 110 °C, detector 150 °C). The volume of biogas produced was measured by water displacement in laboratory experiments (I–V) and by gas volume meters (Gallus 2000, Actaris Technologies AB) in pilot experiments (V).

Volatile fatty acids (VFAs) were measured with a GC equipped with flame-ionisation detector (Perkin Elmer Autosystem XL, PE FFAP column 30 m × 0.32 mm × 25 μm, carrier gas helium, injection port and detector 225 °C, oven 100 to 160 °C (20 °C/min)) in III–IV and with a high performance liquid

chromatograph (Varian Star 9000 HPLC, Biorad column 125–0115, column temperature 65 °C, mobile phase 1mM sulphuric acid 0.8 ml min^{-1}) in V.

TS and VS were determined according to the Standard Methods (APHA 1998). The pilot reactors (V) were equipped with on-line pH and temperature indicators (MiniCHEM-pH Process Monitor, TPS Pty Ltd.), and Metrohm 774 pH-meter was used in other pH measurements. pH of solid materials was measured from a mixture of solid substrate and distilled water (L/S ratio of 2) after homogenising with a kitchen blender. COD and NH_4-N from crop samples were analysed after extraction according to SFS-EN 12457–4 (Finnish Standards Association 2002). The samples for NH_4-N and SCOD determination were filtered with GF50-glass fibre filter papers (Schleicher & Schuell) (I–IV), or alternatively the supernatant from centrifugation (3 000 rpm, 3 min) was used in analyses (V). COD was measured according to the SFS 5504 (Finnish Standards Association 1988) (I–IV) or with Dr Lange cuvette tests (LCK 114 and LCK 914, Dr. Bruno Lange GmbH) (V). NH_4-N and N_{tot} were determined according to the Tecator application note (Perstorp Analytical/Tecator AB 1995) with a Kjeltec system 1002 distilling unit (Tecator AB) (I–IV), or Dr Lange cuvette tests were used for measuring NH_4-N (LCK 303 and LCK 302, Dr. Bruno Lange GmbH) and samples for N_{tot} were analysed colorimetrically using a FIAStar 5000 analyser coupled with a 5027 sampler (Foss Tecator AB) (V).

Extractives were determined by acetone extraction according to the TAPPI Test Method T 280 pm–99 (TAPPI 2000) (IV). For lignin and carbohydrate analyses, the acetone-extracted samples were hydrolysed according to the TAPPI Test Method T 249 cm–00 (TAPPI 2000). Klason lignin content was measured according to the TAPPI Test Method T 222 om–98 (TAPPI 2000). Acid soluble lignin ($lignin_{AS}$) in hydrolysis filtrate was quantified spectroscopically (Beckman DU640 Spectrofotometer) on the basis of UV absorption at 205 nm using an absorptivity value of 110 l g^{-1} cm^{-1}, and $lignin_{tot}$ was calculated as the sum of Klason lignin and $lignin_{AS}$ (I, IV). The monosaccharides obtained (arabinose, galactose, mannose and xylose from the hemicellulose components and glucose from cellulose) were per(trimethylsilyl)ated and analysed with a GC (HP 5890 Series II GC equipped with flame-ionisation detector and a DB-1701 column, 60 m × 0.32 mm, Agilent Technologies, J&W Scientific) (I, IV). Operating conditions were: injection port 290 °C and detector 300 °C. Oven temperature was programmed to begin at 100 °C (held for 2 min), rise 2 °C/min to 185 °C (22 min) and rise 39 °C/min to a final temperature of 280 °C (15 min). Nitrogen was used as carrier gas. Carbon was analysed with elemental analyser EA1110 CHNS-O (CEinstruments) (I). Heat content was analysed as higher heat content with a bomb calorimeter (IKA-Kalorimeter C400, Janke & Kunkel GmbH) (IV). Samples of substrates and solid residues from pilot experiments (V) were analysed at AnalyCen Nordic AB (Lidköping, Sweden) for total carbon (C), crude fibre, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, higher heat content and NH_4-N. Heavy metals (cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), zinc (Zn), arsenic (As), chrome (Cr) and mercury (Hg)) were extracted from the samples by the autoclave digestion method according to EN ISO 1483 (European Committee for Standardization 1997) and analysed by inductively coupled plasma mass spectrometry (ICP-MS) as previously described (Tyler & Olsson 2001) (V).

Methane potentials of substrates were calculated as m^3 CH_4 kg^{-1} VS_{added}, m^3 CH_4 kg^{-1} TS_{added} and m^3 CH_4 t^{-1} ww, minus the methane potential of the inoculum. From these values, the methane potentials per kg $VS_{original}$ (kg VS of fresh crop before storage and addition of storage additive) and per t FW (tons of FW of fresh crop before storage and addition of storage additive) were calculated in storage experiments (II). Methane potentials per hectare were calculated using estimates of biomass yields found in literature (I). Assuming the methane produced could be used a vehicle fuel, the corresponding values for passenger car transport (in km ha^{-1}) were calculated assuming average consumption of 8 m^3 CH_4 100 km^{-1} in passenger cars (I). OLRs and HRTs in CSTRs (III) were calculated on the basis of the daily feedstock additions, and volumetric methane production was calculated as methane production per unit volume of reactor (m^3 CH_4 $m^{-3}_{reactor}$ d^{-1}) (III). Protein content was calculated as 6.25 x N_{tot} whereas organic nitrogen (org-N) was calculated as the difference between N-tot and NH_4-N (IV–V).

4 RESULTS

4.1 Chemical characteristics and methane potentials of potential boreal energy crops and crop residues

The chemical characteristics of the potential energy crops and crop residues were determined (Tables 9–10, Fig. 4). TS content varied a lot, from 9% in rhubarb to 90% in straws, whereas VS/TS-ratio ranged from 0.82 to 0.97. The TS content and the VS/TS ratio of the crops typically increased as the crop matured. However, in timothy-clover grass the TS content decreased as the grass matured, apparently due to the larger amount of clover in the more mature timothy-clover grass. This was also indicated by the higher nitrogen content in the crop from later harvest, contrary to most other crops. Nitrogen content varied from 0.5% TS in Jerusalem artichoke (1st harvest) and straw of oats to 5.2% TS in red clover (1st harvest). Carbon content had lower variation, from 40 to 48% TS. The C/N ratio of the crops varied from 9 (red clover) to 95 (straw of oats), averaging at 28. Legumes and nettle had lowest C/N ratios. Sugar beets and grass silage with 50% clover (V) had the lowest lignin contents (2 and 5% TS, respectively), whereas that of giant knotweed was highest (28% TS) (Tables 9–10). Lignin content was typically lower at earlier harvest, lupine and Jerusalem artichoke being the only exceptions (Table 9).

The methane potentials of crops varied from 0.17 to 0.49 m^3 CH_4 kg^{-1} VS_{added}, most crops having methane potentials in the range 0.3–0.4 m^3 CH_4 kg^{-1} VS_{added} (Tables 13–14, Fig. 4). Rhubarb (1st harvest) had the highest methane potential (0.49 m^3 CH_4 kg^{-1} VS_{added}), and those of whole sugar beets, reed canary grass (2nd harvest), nettle (2nd harvest) and vetch-oat (1st and 2nd harvest) were also quite high (>0.40 m^3 CH_4 kg^{-1} VS_{added}) (Tables 13–14). The lowest methane potentials per VS were acquired for giant knotweed and nettle (1st harvest) (Table 13). With grasses, the methane potentials per VS increased by 3–26% as the crop matured, whereas with legumes they decreased by 2–14%. Among the leafy crops, for Jerusalem artichoke and rhubarb the methane potentials per VS

decreased by 3 and 35%, respectively, as the harvest was postponed, whereas with the other leafy crops the methane potentials per VS increased at the later harvest. The effects of harvest time on methane potentials per VS were most notable with nettle (100% increase at later harvest), giant knotweed (59% increase at later harvest) and rhubarb (35% decrease at later harvest) (Table 13). Red clover, vetch-oat, lupine, Jerusalem artichoke and marrow kale produced very similar patterns of methane production regardless of the stage of maturity. Therefore, in figure 4 the cumulative methane yields of these crops are presented only for crop from first harvest.

When calculated against wet weights, the methane potentials of crops had large variation from 25 to 260 m^3 CH_4 t^{-1} ww. Straws had very high methane potentials per ww (117–260 m^3 CH_4 t^{-1} ww), and those of willow, reed canary grass (2nd harvest) and Jerusalem artichoke (2nd harvest) were also high (>110 m^3 CH_4 t^{-1} ww). Lowest methane potentials per ww were obtained for rhubarb (2nd harvest), nettle (1st harvest), giant knotweed (1st harvest) and sugar beet tops (Tables 13-14). In general, straws and grasses had high methane potentials per ww, whereas those of legumes were generally lower and within the leafy crops there was large variation. The effect of harvest time on methane potentials per ww was remarkable with several crops, and the potentials increased with most crops as the crops matured, timothy-clover grass and rhubarb as the only exceptions. Methane potentials per ww of nettle and giant knotweed increased by over 135% when harvest was postponed to later stage of growth, and increases of 66–71% were evident with reed canary grass, vetch-oat and red clover, whereas the methane potential per ww of rhubarb decreased by 36% when harvest was postponed to the flowering stage (Table 13).

Methane potentials per hectare were calculated using estimates of biomass yields of crops per hectare obtained from the literature (Table 15). Jerusalem artichoke, timothy-clover grass and reed canary grass gave the highest methane potentials of 2 900–5 400 m^3 CH_4 ha^{-1}, corresponding to a gross energy potential of 28–53 MWh and approximately 40 000–60 000 kilometres in passenger car transport per hectare. Due to the apparent low biomass yields per hectare, the methane potential per hectare for straws, lawn and tops of sugar beet remained relatively low (400–1 500 m^3 CH_4 ha^{-1}). Among the potential energy crops the methane potential per hectare remained lowest for rhubarb (Table 15).

TABLE 13 Durations of the batch assays, total methane potentials of the substrates (methane potential of inoculum subtracted) and short-term (30 and 50 d) methane potentials expressed as proportion of the total methane potential (averages of replicates ± standard deviations where applicable).

Substrate	Harvest	Duration of the batch assay	Total methane potential			Short-term methane potential	
		(d)	(m^3 CH_4 kg^{-1} VS_{added})	(m^3 CH_4 kg^{-1} TS_{added})	(m^3 CH_4 t^{-1} ww)	(% of total on day 30)	(% of total on day 50)
GRASSES:							
Timothy-clover grass	1	146	0.37 ±0.02	0.34 ±0.02	85 ±5	70	90
	2	125	0.38 ± 0.00	0.36 ±0.00	72 ±0	49	87
Reed canary grass	1	125	0.34 ± 0.00	0.33 ±0.00	97 ±0	76	88
	2	194	0.43 ± 0.02	0.42 ±0.02	167 ±8	54	71
Lawn	1	124	0.30 ± 0.04	0.27 ±0.04	58 ±8	87	96
LEGUMES:							
Red clover	1	125	0.30 ± 0.06	0.27 ±0.05	41 ±8	61	89
	2	124	0.28 ± 0.06	0.26 ±0.06	68 ±15	66	89
Vetch-oat mixture	1	130	0.41 ± 0.02	0.37 ±0.02	57 ±3	56	89
	2	124	0.40 ± 0.04	0.37 ±0.04	95 ±10	72	89
Lupine	1	140	0.36 ± 0.04	0.33 ±0.04	40 ±4	71	89
	2	125	0.31 ± 0.06	0.29 ±0.06	41 ±8	74	92
LEAFY CROPS:							
Jerusalem artichoke	1	150	0.37 ± 0.06	0.34 ±0.06	93 ±15	54	90
	2	140	0.36 ± 0.04	0.34 ±0.04	110 ±12	46	86
Giant knotweed	1	125	0.17 ± 0.08	0.16 ±0.08	32 ±15	78	80
	2	164	0.27 ± 0.00	0.25 ±0.00	76 ±0	73	78
Nettle	1	130	0.21 ± 0.00	0.17 ±0.00	25 ±0	93	99
	2	125	0.42 ± 0.06	0.36 ±0.05	60 ±9	75	91
Rhubarb	1	107	0.49 ± 0.03	0.42 ±0.03	40 ±2	85	94
	2	107	0.32 ± 0.02	0.28 ±0.02	25 ±2	89	99
Marrow kale	1	150	0.31 ± 0.02	0.28 ±0.02	37 ±2	21	73
	2	140	0.32 ± 0.02	0.29 ±0.02	38 ±2	20	77
Tops of sugar beet		139	0.34 ± 0.00	0.29 ±0.00	34 ±0	47	88
STRAWS:							
Straw of oats		150	0.32 ± 0.02	0.29 ±0.02	260 ±16	44	76
Straw of rapeseed		154	0.24 ± 0.02	0.22 ±0.02	199 ±17	60	69

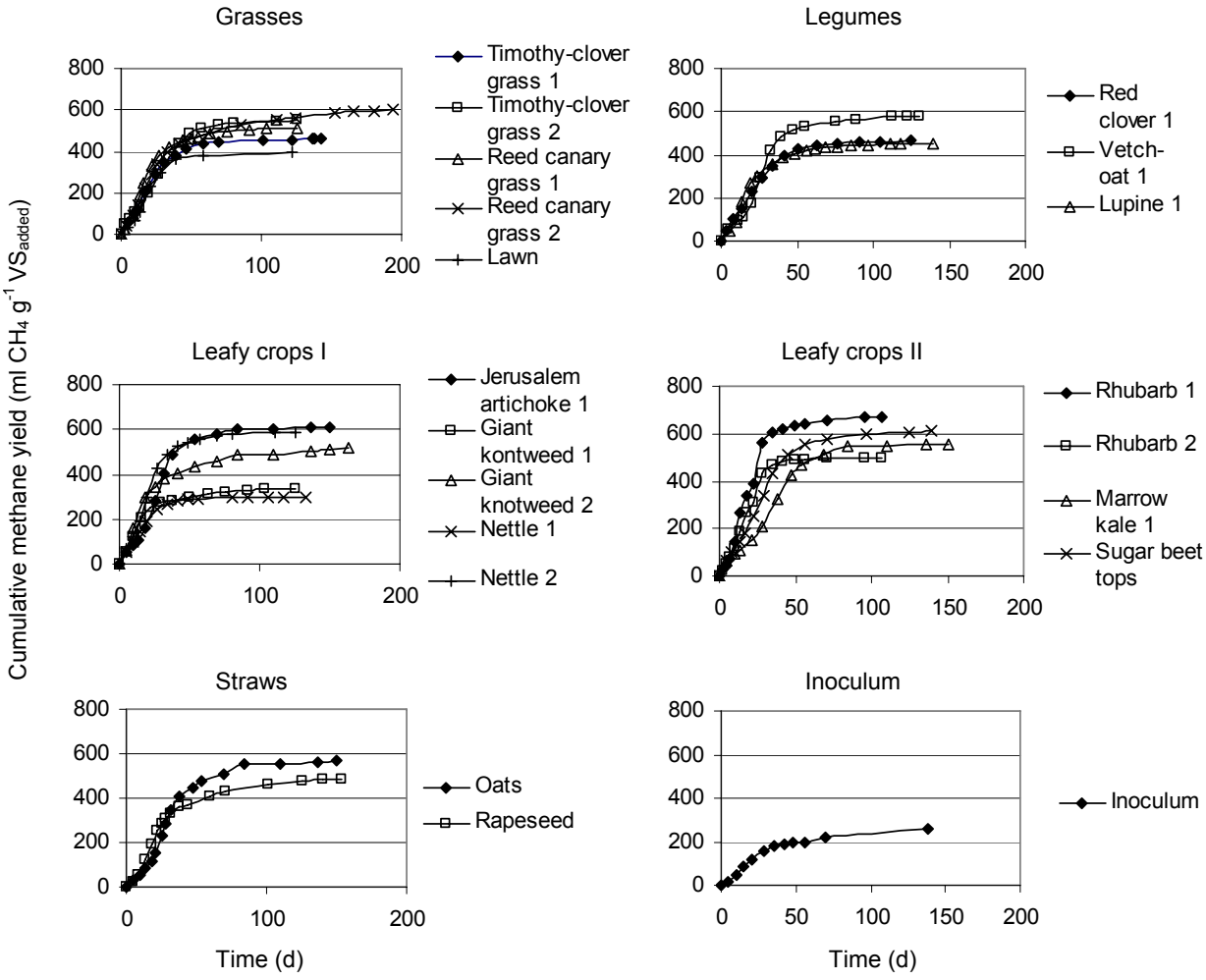

FIGURE 4 Mean cumulative methane production per VS_{added} of crops in the screening experiment (1 = 1st harvest, 2 = 2nd harvest).

TABLE 14 Methane potentials of substrates used in storage and reactor experiments (average values of replicates ± standard deviations, where applicable).

Substrate	Duration of the batch assay (d)	Total methane potential (m^3 CH_4 kg^{-1} VS_{added})	Total methane potential (m^3 CH_4 kg^{-1} TS_{added})	Total methane potential (m^3 CH_4 t^{-1} ww)	Short-term methane potential (m^3 CH_4 kg^{-1} VS_{added})	Short-term methane potential (% of total)
Grass (fresh) (II)	42	0.231 ±0.030	0.213 ±0.028	64 ±8	n.d.	n.d.
Grass silage (III)	94	0.306 ±0.003	0.284 ±0.003	74 ±1	0.206 ±0.002[a]	67[a]
Grass silage (IV)	94	0.300 ±0.003	0.279 ±0.003	72 ±1	0.262 ±.0.003[b]	87[b]
Grass silage (V)	64	0.372 ±.0.015	0.326 ±0.013	104 ±4	0.358 ±0.012[b]	96[b]
Sugar beet tops (II)	42	0.310 ±0.030	0.252 ±0.024	28 ±3	n.d.	n.d.
Sugar beet tops (III)	87	0.353 ±0.018	0.283 ±0.014	29 ±1	0.181 ±0.009[a]	51[a]
Sugar beet (V)	90	0.448 ±0.018	0.397 ±0.016	80 ±3	0.419 ±0.024[b]	94[b]
Oat straw (III)	94	0.203 ±0.025	0.185 ±0.023	117 ±14	0.138 ±0.017[a]	68[a]
Willow (V)	139	0.289 ±0.007	0.284 ±0.007	140 ±3	0.155 ±0.005[b]	54[b]
Cow manure (III)	92	0.233 ±0.020	0.190 ±0.016	12 ±1	0.204 ±0.016[a]	88[a]

[a] Methane potential after 20 d of incubation, [b] Methane potential after 30 d of incubation.

4.2 Effect of storage on methane potential of energy crops and crop residues

Grass and sugar beet tops were stored as silage with and without additives (FA, enzymes, LAB, mixed culture) for 3 and 6 months at 20 and 5 °C in order to determine the effect of storage on the chemical characteristics and methane potentials of these substrates (II). The pH of fresh crops in both cases was 6.4, whereas after storage it ranged from 4.2 to 6.6 with grass and from 3.8 to 5.8 with sugar beet tops, the highest values often occurring after storage at 5 °C (Table 16). The TS and VS concentrations of stored crops were in general lower than those of fresh materials. Concentrations of N_{tot} in fresh crops were 39 mg g^{-1} TS in grass and 28 mg g^{-1} TS in sugar beet tops, and they changed little during storage (33–43 mg g^{-1} TS in stored grass and 26–38 mg g^{-1} TS in stored sugar beet tops, Table 16). However, the proportions of NH_4-N from N_{tot} were higher in stored crops than in fresh crops (2.0% in fresh grass and 0.7% in fresh sugar beet tops), ranging from 3.7 to 9.5% in stored grass and from 3.9 to 24.3% in stored sugar beet tops, the highest values occurring in crops stored with the mixed culture (Table 16).

TABLE 15 Annual dry matter yields of crops per hectare in boreal growing conditions, methane and gross energy potentials per hectare, and corresponding passenger car transport in km ha^{-1}.

Substrate	Yield (t TS ha^{-1})	Methane potential (m^3 CH_4 $ha^{-1}a^{-1}$)	Gross energy potential (MWh ha^{-1} a^{-1})	Passenger car transport (1000 km ha^{-1} a^{-1})
GRASSES:				
Timothy-clover grass	8–11 [a]	2 900 – 4 000	28–38	36–50
Reed canary grass	9–10 [b]	3 800 – 4 200	37–41	47–53
Lawn	2 [c]	500	5	7
LEGUMES:				
Red clover	5–7 [a]	1 400 – 1 900	13–18	17–24
Vetch-oat mixture	5–7 [d]	1 900 – 2 600	18–25	23–32
Lupine	4–7 [e]	1 300 – 2 300	13–22	17–29
LEAFY CROPS:				
Jerusalem artichoke	9–16 [f]	3 100 – 5 400	30–53	38–68
Giant knotweed	15 [g]	3 800	36	47
Nettle	6–10 [h]	2 200 – 3 600	21–35	27–45
Rhubarb	2–4 [i]	800 – 1 700	8–16	11–21
Marrow kale	6–8 [j]	1 700 – 2 300	17–23	22–29
Tops of sugar beet	3–5 [j]	900 – 1 500	8–14	11–18
STRAWS:				
Straw of oats	2 [b, j]	600	6	7
Straw of rapeseed	2 [b]	400	4	6

[a] Kangas et al. 2004, [b] Sankari 1993, [c] Tenhunen & Pelkonen 1987, [d] Kiljala & Isolahti 2003, [e] Aniszewski 1993, [f] Häggblom 1988, [g] yield in United Kindgom, Callaghan et al. 1985a, [h] Galambosi 1995, [i] Nissi 2003, [j] Hyytiäinen et al. 1999.

The methane potentials of fresh and stored substrates were determined in 42 d batch assays at 35 °C (Fig. 5–6). There was a lag of 3 days in methane production in batch assays with fresh grass and inoculum only, but in all the other batch assays methane production started immediately (Fig. 5–6).

When grass was stored without additives, 19–24% of VS was lost, but the effect on post storage methane potential per VS_{added} was minor. Taking into account the losses of VS during storage, the methane potentials calculated per $VS_{original}$ of grass decreased by 17–39% after storage without additives, decreasing with time (Table 17, Fig. 7). With sugar beet tops the losses of VS during storage without additives were higher, ranging between 25 and 34%. However, the methane potentials per VS_{added} of sugar beet tops increased during storage without additives, and the losses in methane potential calculated per $VS_{original}$ remained low (0–13%) (Table 17, Fig. 7).

The addition of storage additives, with the exception of LAB, increased the methane potential per VS_{added} of grass by 4–22% (Table 17, Fig. 5). When grass was stored with additives, losses of VS ranged from 0 to 24%, storage with the mixed culture being particularly efficient in conserving the grass VS, while the highest losses of VS occurred in storage with LAB (Table 17). Storage with FA, enzymes and LAB increased the grass methane potential per VS_{added} by up to 11, 8 and 41%, respectively, compared with the mixture of grass and additive at the beginning of storage, whereas it decreased by 11–15% during storage with the mixed culture (Table 17). However, when the losses in VS were taken into account, storage with LAB was the only treatment to increase the grass methane potential per $VS_{original}$ (by 5–11%) compared to the mixture of grass and additive

TABLE 16 Chemical characteristics of grass and sugar beet tops before and after storage.

Storage method	Duration	T	Grass						Sugar beet tops					
			pH	TS	VS	N_{tot}	NH_4-N		pH	TS	VS	N_{tot}	NH_4-N	
	(months)	(° C)		(%ww)	(%ww)	(mg g^{-1} TS)	(mg g^{-1} TS)	(% N_{tot})		(%ww)	(%ww)	(mg g^{-1} TS)	(mg g^{-1} TS)	(% N_{tot})
No additive	0[a]	-	6.4	30.2	27.9	39.2	0.8	2.0	6.4	11.2	9.1	27.7	0.2	0.7
	3	20	5.0	24.4	22.7	37.8	1.9	5.0	4.0	9.2	6.9	27.5	1.6	5.8
	6	20	4.5	24.9	22.7	37.7	2.8	7.4	4.1	8.4	6.1	32.9	2.8	8.5
	6	5	6.6	23.7	21.3	42.3	2.7	6.4	4.7	8.7	6.4	30.3	1.6	5.3
FA	0[b]	-	4.3	32.7	30.5	n.a.	n.a.	n.a.	3.0	11.5	9.1	n.a.	n.a.	n.a.
	3	20	4.4	30.4	28.4	32.5	1.2	3.7	3.8	9.5	7.2	25.7	1.6	6.2
	6	20	4.6	25.9	23.5	36.6	3.3	9.0	3.9	8.4	6.1	30.1	4.3	14.3
	6	5	5.0	28.5	26.1	39.3	1.9	4.8	5.7	9.1	6.8	25.5	1.0	3.9
Enzyme	0[b]	-	6.5	29.9	27.7	n.a.	n.a.	n.a.	6.3	10.9	8.6	n.a.	n.a.	n.a.
	3	20	4.3	27.7	25.6	34.9	1.6	4.6	4.0	8.1	5.9	31.8	2.8	8.8
	6	20	4.4	25.9	23.5	39.6	2.2	5.6	3.9	8.0	5.8	29.2	3.0	10.3
	6	5	4.8	27.1	24.8	41.6	1.8	4.3	5.8	7.7	5.5	28.8	2.2	7.6
LAB	0[b]	-	6.3	31.6	30.2	n.a.	n.a.	n.a.	6.5	10.8	8.6	n.a.	n.a.	n.a.
	3	20	4.2	26.2	24.1	36.6	1.5	4.1	3.8	9.4	7.2	30.7	1.6	5.2
	6	20	4.4	25.5	23.2	39.7	2.5	6.3	3.9	8.9	6.7	25.8	2.4	9.3
	6	5	5.0	26.9	24.6	39.6	1.5	3.8	4.3	7.7	5.6	29.5	1.8	6.1
Mixed culture	0[b]	-	8.0	22.0	20.2	n.a.	n.a.	n.a.	8.0	9.9	7.8	n.a.	n.a.	n.a.
	3	20	4.5	22.2	20.3	42.2	2.8	6.6	4.6	7.4	5.4	38.1	6.5	17.1
	6	20	5.0	20.6	18.4	43.2	4.1	9.5	5.0	7.0	4.9	37.1	9.0	24.3
	6	5	5.0	20.0	18.1	42.1	4.0	9.5	4.3	8.2	6.1	37.4	3.5	9.4

[a] Fresh crop, [b] After addition of storage additive, n.a. = not analysed.

TABLE 17 Losses of grass and sugar beet top VS during storage and methane potentials of fresh and stored grass and sugar beet tops (averages of replicates ± standard deviations, where applicable).

Storage method	Dura-tion	T	Grass				Sugar beet tops				
			Loss of VS	Methane potential [c]			Loss of VS	Methane potential [c]			
	(months)	(° C)	(%)	($m^3\,kg^{-1}$ VS_{added})	($m^3\,t^{-1}$ ww)	($m^3\,kg^{-1}$ $VS_{original}$)[d]	($m^3\,t^{-1}$ FW)[d]	(%)	($m^3\,kg^{-1}$ VS_{added})	($m^3\,t^{-1}$ ww)	($m^3\,kg^{-1}$ $VS_{original}$)[d]	($m^3\,t^{-1}$ FW)[d]
No additive	0[a]	-	-	0.23 ±0.03	64.2 ±8.4	0.23 ±0.03	64.2 ±8.4	-	0.31 ±0.03	28.2 ±2.7	0.31 ±0.03	28.2 ±2.7
	3	20	19	0.23 ±0.03	52.2 ±6.8	0.19 ±0.02	51.8 ±6.8	25	0.36 ±0.01	24.8 ±0.7	0.27 ±0.01	24.6 ±0.7
	6	20	20	0.18 ±0.03	40.9 ±6.8	0.14 ±0.02	40.4 ±6.7	34	0.47 ±0.02	28.7 ±1.2	0.31 ±0.01	28.2 ±1.2
	6	5	24	0.23 ±0.02	49.0 ±4.3	0.17 ±0.02	48.6 ±4.2	31	0.43 ±0.02	27.5 ±1.3	0.30 ±0.01	27.2 ±1.3
FA	0[b]	-	-	0.28 ±0.00	85.4 ±0.0	0.31 ±0.00	85.9 ±0.0	-	0.29 ±0.06	26.4 ±5.5	0.29 ±0.06	26.2 ±5.4
	3	20	8	0.28 ±0.01	79.5 ±2.8	0.28 ±0.01	79.0 ±2.8	21	0.33 ±0.01	23.8 ±0.7	0.26 ±0.01	23.7 ±0.7
	6	20	24	0.31 ±0.02	72.9 ±4.7	0.26 ±0.02	72.0 ±4.6	34	0.52 ±0.03	31.7 ±1.8	0.34 ±0.02	31.2 ±1.8
	6	5	16	0.28 ±0.03	73.1 ±7.8	0.26 ±0.03	72.1 ±7.7	26	0.37 ±0.06	25.2 ±4.1	0.27 ±0.04	24.9 ±4.0
Enzyme	0[b]	-	-	0.24 ±0.04	66.5 ±11.1	0.24 ±0.04	67.1 ±11.2	-	0.28 ±0.04	24.1 ±3.4	0.27 ±0.04	24.2 ±3.5
	3	20	8	0.24 ±0.04	61.4 ±10.2	0.22 ±0.04	61.5 ±10.3	33	0.38 ±0.00	22.4 ±0.0	0.24 ±0.00	22.1 ±0.0
	6	20	16	0.26 ±0.00	61.1 ±0.0	0.22 ±0.00	60.9 ±0.0	34	0.43 ±0.04	24.9 ±2.3	0.27 ±0.03	24.7 ±2.3
	6	5	11	0.24 ±0.03	59.5 ±7.4	0.21 ±0.03	59.6 ±7.5	37	0.36 ±0.06	19.8 ±3.3	0.22 ±0.04	19.7 ±3.3
LAB	0[b]	-	-	0.17 ±0.01	51.3 ±3.0	0.19 ±0.01	51.7 ±3.0	-	0.27 ±0.03	23.2 ±2.6	0.25 ±0.03	23.1 ±2.6
	3	20	21	0.24 ±0.03	57.8 ±7.2	0.21 ±0.03	57.8 ±7.2	16	0.32 ±0.02	23.0 ±1.4	0.25 ±0.02	22.9 ±1.4
	6	20	24	0.24 ±0.04	55.7 ±9.3	0.20 ±0.03	55.2 ±9.2	23	0.44 ±0.01	29.5 ±0.7	0.32 ±0.01	29.0 ±0.7
	6	5	20	0.24 ±0.05	59.0 ±12.3	0.21 ±0.04	58.7 ±12.2	35	0.41 ±0.04	23.0 ±2.2	0.25 ±0.02	22.6 ±2.2
Mixed culture	0[b]	-	-	0.27 ±0.01	54.5 ±2.0	0.24 ±0.01	68.2 ±2.5	-	0.25 ±0.04	19.5 ±3.1	0.27 ±0.04	24.3 ±3.9
	3	20	0	0.23 ±0.05	46.7 ±10.2	0.21 ±0.05	58.0 ±12.6	32	0.38 ±0.01	20.5 ±0.5	0.28 ±0.01	25.3 ±0.7
	6	20	10	0.23 ±0.02	42.3 ±3.7	0.19 ±0.02	52.0 ±4.5	39	0.51 ±0.05	25.0 ±2.5	0.33 ±0.03	30.3 ±3.0
	6	5	11	0.24 ±0.03	43.4 ±5.4	0.19 ±0.02	53.7 ±6.7	22	0.45 ±0.05	27.5 ±3.1	0.37 ±0.04	34.0 ±3.8

[a] Fresh crop, [b] After addition of storage additive, n.a. = not analysed.

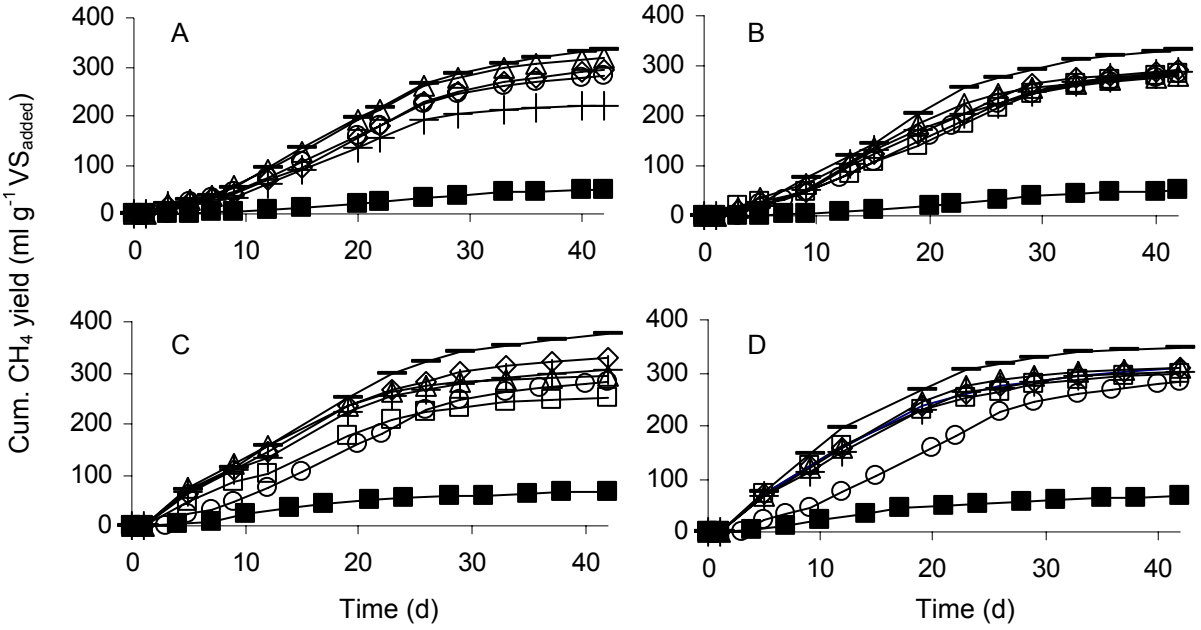

FIGURE 5 Mean cumulative methane production per VS_{added} in methane potential assays with grass. A = after addition of storage additives, B = after storage for 3 months at 20 °C, C = after storage for 6 months at 20 °C, and D = after storage for 6 months at 5 °C. ■ = inoculum, ○ = fresh crop, □ = without additives, ▬ = with FA, ◊ = with enzymes, + = with LAB, Δ = with the mixed culture.

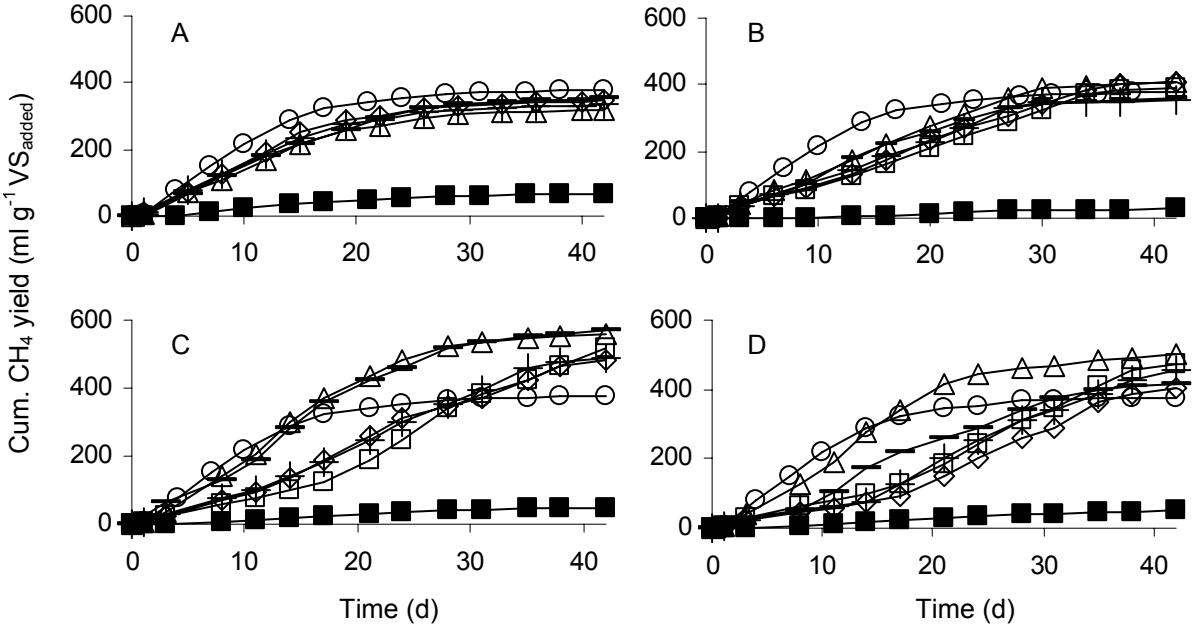

FIGURE 6 Mean cumulative methane production per VS_{added} in methane potential assays with sugar beet tops. A = after addition of storage additives, B = after storage for 3 months at 20 °C, C = after storage for 6 months at 20 °C, and D = after storage for 6 months at 5 °C. ■ = inoculum, ○ = fresh crop, □ = without additives, ▬ = with FA, ◊ = with enzymes, + = with LAB, Δ = with the mixed culture.

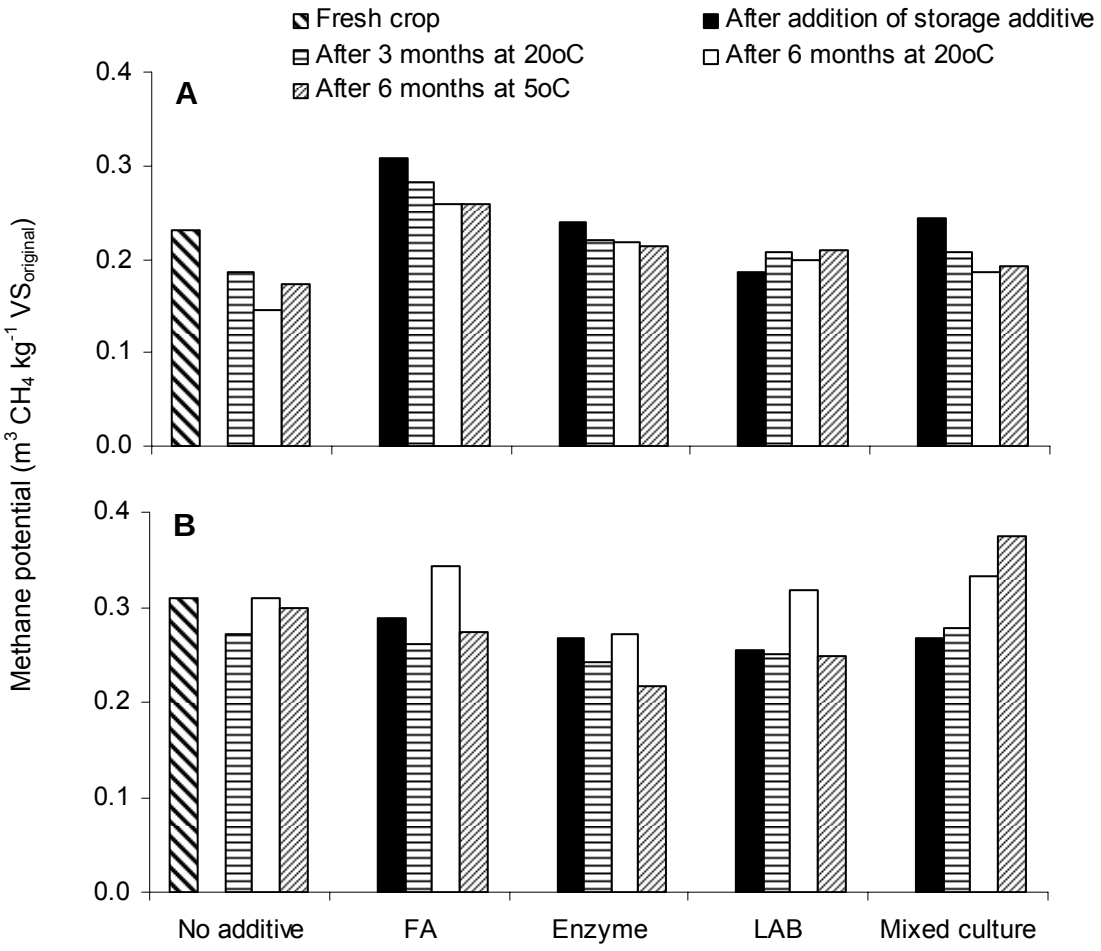

FIGURE 7 Methane potentials of fresh and stored grass (A) and sugar beet tops (B) expressed as m^3 CH_4 kg^{-1} $VS_{original}$.

at the beginning of storage, whereas with the other treatments it decreased during storage by 8–21% (Table 17, Fig. 7). As the initial addition of LAB decreased the methane potential of grass, there was no overall improvement in methane potential after storage of grass with LAB as compared to the fresh substrate (Table 17, Fig. 7). However, many other storage conditions improved the methane potential per VS_{added} of grass as compared to the fresh substrate, the highest increase occurring after storage with FA (Table 17, Fig. 5). Taking into account the losses of VS during storage, the losses in methane potential compared with fresh grass ranged from 4 to 17%, with the exception of storage with FA, which increased the methane potential per $VS_{original}$ of grass by 13–22% compared with that of fresh grass and by 47–86% compared with grass stored without additives (Table 17, Fig. 7).

With sugar beet tops, the addition of storage additives decreased the methane potential per VS_{added} by 6–19% (Table 17, Fig. 6). The methane potential per VS_{added} of sugar beet tops increased significantly during storage with additives compared with the mixture of sugar beet tops and additive at the beginning of storage (by 14–79, 29–54, 19–63 and 52–104% during storage with

FA, enzymes, LAB and mixed culture, respectively) (Table 17). The losses of VS during the storage of sugar beet tops, regardless of whether or not additives were used, were high (16–39%) and, therefore, the increase in the methane potential per $VS_{original}$ of sugar beet tops during storage with additives compared with that at the beginning of storage was up to 17, 28 and 37% after storage with FA, LAB and mixed culture, respectively) (Table 17, Fig. 7). Compared with the fresh substrate, the methane potentials per VS_{added} of sugar beet tops increased during storage in all storage conditions, storage with FA and the mixed culture (6 months at 20 °C) showing particular efficacy in improving the methane potentials per VS_{added} of sugar beet tops (65–68% increase when compared to the fresh crop) (Table 17, Fig. 6). Despite the high losses of VS during storage of sugar beet tops, storage with the mixed culture (6 months at 5 and 20 °C), with FA (6 months at 20 °C) and with LAB (6 months at 20 °C) increased the methane potential per $VS_{original}$ of this substrate by 6–19, 10 and 3%, respectively, when compared with the fresh crop or crop stored without additives (Table 17, Fig. 7).

When grass was stored with additives, the methane potential per $VS_{original}$ decreased slightly with time, whereas temperature had little influence on it. However, during storage without additives, the effect of duration of storage on the methane potential of grass was more pronounced, losses in methane potential being higher after storage at 20 °C (Table 17, Fig. 7). The methane potential per $VS_{original}$ of sugar beet tops was always higher after storage for 6 months than after storage for 3 months, and the methane potential in sugar beet tops was better conserved at 20 °C in storage with FA, enzymes and LAB as well as without additives, whereas in storage with the mixed culture, the methane potential of sugar beet tops was better conserved at 5 °C (Table 17, Fig. 7).

4.3 Co-digestion of energy crops and crop residues with cow manure

Four parallel laboratory CSTRs were operated to evaluate co-digestion of the plant materials with manure (III). Initially, all reactors were fed for 27 days with manure at OLR of 2 kg VS m^{-3} d^{-1} and HRT of 20 d. Subsequently, one reactor (R1) was run for an additional 28 days with manure alone whereas in the other reactors the feeding of crops along with manure was initiated by replacing 10% of the feedstock VS with crops (sugar beet tops in R2, grass in R3 and straw in R4), while maintaining constant OLR and HRT. The proportion of crops in the feedstock was then gradually increased up to 40% of the feedstock VS (Fig. 8 and 9, Tables 18–20) and, finally, the OLRs of the reactors co-digesting manure with grass and straw (R3 and R4) were increased first to 3 and then 4 kg VS m^{-3} d^{-1}, decreasing the HRTs to 18 and 16 d, respectively (Fig. 8 and 9, Tables 19–20).

During the first 27 days when all the reactors were fed simultaneously with manure, reactors R1, R3 and R4 showed nearly identical specific methane yields (0.151 to 0.155 m^3 CH_4 kg^{-1} VS_{added}, Tables 18–20, Fig. 8) and reactor R2 a

slightly lower yield (0.133 m^3 CH_4 kg^{-1} VS_{added}, Table 18, Fig. 8). The VS removals ranged from 20 to 26% (Tables 18–20). The initiation of the feeding of crops along with manure (day 28 in reactors R2–R4) led to a temporary decrease in specific methane yield, but as the proportion of crop in the feedstock was increased, the specific methane yields and VS removals also increased (Fig. 8, Tables 18–20). The highest specific methane yield was obtained when the proportion of crop in the feedstock was 30% (feeding regime 4) (0.229 m^3 CH_4 kg^{-1} VS_{added} in co-digestion with sugar beet tops (R2), 0.268 m^3 CH_4 kg^{-1} VS_{added} in co-digestion with grass (R3) and 0.213 m^3 CH_4 kg^{-1} VS_{added} in co-digestion with straw (R4)) (Fig. 8, Tables 18–20). During this feeding regime, the volumetric methane productions were 65, 58 and 16% higher in the reactors co-digesting manure with sugar beet tops (R2), grass (R3) and straw (R4), respectively, compared with digestion of manure alone (Fig. 8). Increasing the proportion of crop further to 40% decreased the specific methane yields by 4–12%. The VS removals ranged from 28 to 49% in co-digestion with sugar beet tops (R2), from 41 to 53 in co-digestion with grass (R3), and from 27 to 43% in co-digestion with straw (R4). In reactors co-digesting manure with sugar beet tops (R2) and grass (R3), the VS removals increased as the proportion of crop in the feedstock increased, whereas in the reactor co-digesting manure with straw (R4), the removal was highest during feeding with 20% of straw in the feedstock (Tables 18–20).

Operation of the reactors co-digesting manure with grass (R3) and straw (R4) was continued from day 204 onwards by increasing the OLRs from 2 to 3 and eventually to 4 kg VS m^{-3} d^{-1}, while maintaining the 40% VS proportion of crop in the feedstock. Increasing the OLR from 2 to 3 kg VS m^{-3} d^{-1} decreased the specific methane yield in co-digestion of manure with grass (R3) from 0.250 to 0.233 m^3 CH_4 kg^{-1} VS_{added}, but in co-digestion with straw (R4) there was little change (Fig. 8, Tables 19–20). Further increase in OLR to 4 kg VS m^{-3} d^{-1} decreased the specific methane yield to 0.186 and 0.157 m^3 CH_4 kg^{-1} VS_{added} in co-digestion with grass (R3) and straw (R4), respectively. At OLRs 3 and 4 kg VS m^{-3} d^{-1}, the VS removals in co-digestion with grass (R3) and straw (R4) amounted to 52-53 and 40%, respectively (Tables 19–20).

TS removals in the digestion of manure alone ranged from 12 to 19%, but increased when crops were included in the feedstock, ranging then from 20 to 48%, being lowest in co-digestion with straw (Tables 18–20). In all the reactors, formation of a crust layer in the upper part of the liquid space was observed from feeding regime 2 (20% of crop in the feedstock) onwards, the layer increasing in depth as the experiment proceeded and thickest in co-digestion of straw (R4), followed by co-digestion with grass (R3), and least apparent in co-digestion with sugar beet tops (R2).

During all runs, NH_4-N accounted for 33–48% (0.5–1.0 g l^{-1}) of N_{tot} in the digested material, whereas in the feedstock, the proportion of NH_4-N of N_{tot} varied between 30–38%. On average, the proportion of NH_4-N of N_{tot} increased by 26, 25 and 14% during co-digestion of manure with sugar beet tops, grass and straw, respectively. The pH of the digestates remained between 7.2 and 7.8 and the VFA concentrations were < 0.3 g l^{-1} in all digestates throughout the run (VFAs measured approximately once per week, data not shown), while values for SCOD ranged from 5 to 12 g l^{-1} (Fig. 9, Tables 18–20). As the proportion of

crop in the feedstock was increased, the values for TS, VS and SCOD of the digestates decreased (Fig. 9, Tables 18–20). However, as the OLR in the reactors digesting manure with grass (R3) and straw (R4) was increased, the values for these parameters increased. Also, a slight increase in digestate ammonia concentrations was observed at the higher OLRs (Fig. 9, Tables 19–20).

The post-methanation potentials of the digestates were measured on several occasions during the runs (Table 21). Post-methanation of the digestates in batch assays incubated for 100 d at 5, 20 and 35 °C yielded 0.001–0.009, 0.073–0.120 and 0.133–0.197 m^3 CH_4 kg^{-1} digestate VS_{added}, respectively. Differences in the post-methanation potentials measured during feeding regimes with 30 and 40% of crop in the feedstock were small, but increasing the OLR from 2 to 4 kg VS m^{-3} d^{-1} led to an increase of 30–37% in the post-methanation potentials of the digestates as measured at 35 °C. The digestate from co-digestion of manure and straw (R4) had the highest post-methanation potential, which reached 0.197 m^3 CH_4 kg^{-1} VS_{added} and 7.7 m^3 CH_4 t^{-1} ww at 35 °C (Table 21).

TABLE 18 Operational conditions, feedstock and digestate characteristics, and methane production in the CSTRs fed with cow manure (R1) and cow manure with sugar beet tops (R2). Feedstock and digestate characteristics and methane production were calculated as averages (± standard deviations, where applicable) of the measurements during the last two weeks of each feeding regime.

Substrate (Reactor)		*Cow manure (R1)*		*Cow manure and sugar beet tops (R2)*			
Feeding regime		1	1	2	3	4	5
Share of crop	% VS	0	0	10	20	30	40
	% ww	0	0	5	10	15	19
OLR	kg VS m^{-3} d^{-1}	2	2	2	2	2	2
HRT	d	20	20	20	20	20	20
Duration	d	0–55	0–27	28–56	57–83	84–143	144–190
	HRT	2.8	1.4	1.4	1.4	3.0	2.3
Feedstock							
TS	%	4.9 ±0.1	4.9 ±0.1	4.9 ±0.1	5.0 ±0.1	5.0 ±0.1	5.0 ±0.1
VS	%	4.0 ±0.1	4.0 ±0.1	4.0 ±0.1	4.0 ±0.1	4.0 ±0.1	4.0 ±0.1
SCOD	g l^{-1}	11.5 ±1.5	11.5 ±1.5	11.7 ±1.4	11.8 ±1.7	12.0 ±2.2	12.2 ±2.9
NH_4-N	g l^{-1}	0.8 ±0.1	0.8 ±0.1	0.7 ±0.1	0.6 ±0.1	0.6 ±0.1	0.5 ±0.1
N_{tot}	g l^{-1}	2.1 ±0.2	2.1 ±0.2	1.9 ±0.1	1.8 ±0.2	1.7 ±0.0	1.6 ±0.3
Digestate							
TS	%	4.0 ±0.2	4.0 ±0.1	3.9 ±0.2	3.4 ±0.3	3.1 ±0.2	2.9 ±0.1
VS	%	3.0 ±0.2	3.0 ±0.1	2.9 ±0.2	2.5 ±0.2	2.2 ±0.2	2.1 ±0.1
SCOD	g l^{-1}	11.6 ±0.7	11.1 ±2.5	9.5 ±1.2	9.4 ±0.5	6.1 ±0.6	5.9 ±0.6
NH_4-N	g l^{-1}	1.0 ±0.1	1.0 ±0.1	0.8 ±0.0	0.8 ±0.0	0.7 ±0.1	0.7 ±0.0
N_{tot}	g l^{-1}	2.0 ±0.1	2.4 ±0.0	1.9 ±0.0	1.9 ±0.1	1.7 ±0.0	1.7 ±0.1
pH		7.5 ±0.1	7.6 ±0.1	7.5 ±0.1	7.4 ±0.1	7.4 ±0.1	7.3± 0.1
TS removal [1]	%	19	18	22	31	38	41
VS removal [1]	%	25	26	28	38	45	49
CH_4 conc.	%	50 ±7	49 ±2	47 ±2	53 ±2	56 ±1	55 ±2
Specific CH_4 yield	m^3 kg^{-1} VS_{added}	0.155 ±0.026	0.133 ±0.017	0.149 ±0.012	0.200 ±0.016	0.229 ±.0.054	0.220 ±0.030
	m^3 t^{-1} ww	6.2 ±1.0	5.3 ±0.7	6.0 ±0.5	8.0 ±0.6	9.2 ±2.2	8.8 ±1.2
% of total CH_4 potential in substrates [1]		67	57	61	78	85	78
% of short-term CH_4 potential in substrates [1]		76	65	74	100	116	112

[1] Calculated on basis of average values

TABLE 19 Operational conditions, feedstock and digestate characteristics, and methane production in the CSTR fed with cow manure and grass silage (R3). Feedstock and digestate characteristics and methane production were calculated as averages (± standard deviations, where applicable) of the measurements during the last two weeks of each feeding regime.

Feeding regime		1	2	3	4	5	6	7
Share of crop	% VS	0	10	20	30	40	40	40
	% ww	0	2	3	5	7	9	11
OLR	kg VS m^{-3} d^{-1}	2	2	2	2	2	3	4
HRT	d	20	20	20	20	20	18	16
Duration	d	0–27	28–55	56–84	85–141	142–203	204–266	267–318
	HRT	1.4	1.4	1.4	2.8	3.1	3.4	3.2
Feedstock								
TS	%	4.9 ±0.1	4.9 ±0.1	4.8 ±0.1	4.7 ±0.1	4.7 ±0.1	6.3 ±0.1	7.5 ±0.1
VS	%	4.0 ±0.1	4.0 ±0.1	4.0 ±0.1	4.0 ±0.1	4.0 ±0.1	5.4 ±0.1	6.4 ±0.1
SCOD	g l^{-1}	11.5 ±1.5	11.3 ±1.4	11.2 ±1.6	11.0 ±2.0	10.8 ±2.6	14.6 ±2.1	17.3 ±1.1
NH_4-N	g l^{-1}	0.8 ±0.1	0.7 ±0.1	0.6 ±0.1	0.6 ±0.1	0.5 ±0.1	0.7 ±0.1	0.8 ±0.1
N_{tot}	g l^{-1}	2.1 ±0.2	1.9 ±0.1	1.8 ±0.2	1.7 ±0.0	1.5 ±0.3	2.1 ±0.1	2.4 ±0.1
Digestate								
TS	%	4.1 ±0.1	3.2 ±0.3	3.2 ±0.4	3.1 ±0.2	2.8 ±0.1	3.3 ±0.1	4.0 ±0.1
VS	%	3.0 ±0.1	2.3 ±0.3	2.3 ±0.3	2.3 ±0.2	2.2 ±0.1	2.6 ±0.1	3.1 ±0.1
SCOD	g l^{-1}	11.6 ±1.2	10.3 ±0.4	9.1 ±0.5	8.2 ±1.0	7.0 ±0.5	8.4 ±0.7	9.3 ±0.8
NH_4-N	g l^{-1}	1.0 ±0.1	0.9 ±0.1	0.8 ±0.0	0.7 ±0.1	0.7 ±0.1	0.7 ±0.0	0.9 ±0.1
N_{tot}	g l^{-1}	2.4 ±0.2	1.9 ±0.3	1.7 ±0.1	1.7 ±0.0	1.8 ±0.3	1.8 ±0.1	2.3 ±0.1
pH		7.6 ±0.1	7.5 ±0.1	7.5 ±0.1	7.4 ±0.1	7.3 ±0.1	7.4 ±0.1	7.6 ±0.1
TS removal [1]	%	17	34	34	35	41	48	47
VS removal [1]	%	26	41	42	43	46	53	52
CH_4 conc.	%	50 ±4	50 ±2	50 ±2	53 ±2	52 ±2	54 ±2	53 ±3
Specific CH_4 yield	m^3 kg^{-1} VS_{added}	0.151 ±0.048	0.143 ±0.016	0.178 ±0.009	0.268 ±0.029	0.250 ±0.016	0.233 ±0.014	0.186 ±0.023
	m^3 t^{-1} ww	6.0 ±1.9	5.7 ±0.6	7.1 ±0.4	10.7 ±1.2	10.0 ±0.6	12.6 ±0.8	11.9 ±1.5
% of total CH_4 potential in substrates [1]		65	62	72	105	95	89	71
% of short-term CH_4 potential in substrates [1]		74	73	87	131	122	114	91

[1] Calculated on basis of average values

TABLE 20 Operational conditions, feedstock and digestate characteristics, and methane production in the CSTR fed with cow manure and straw (R4). Feedstock and digestate characteristics and methane production were calculated as averages (± standard deviations, where applicable) of the measurements during the last two weeks of each feeding regime.

Feeding regime		1	2	3	4	5	6	7
Share of crop	% VS	0	10	20	30	40	40	40
	% ww	0	1	1	2	3	4	4
OLR	kg VS m^{-3} d^{-1}	2	2	2	2	2	3	4
HRT	d	20	20	20	20	20	18	16
Duration	d	0–27	28–55	56–84	85–141	142–203	204–266	267–318
	HRT	1.4	1.4	1.4	2.8	3.1	3.4	3.2
Feedstock								
TS	%	4.9 ±0.1	4.9 ±0.1	4.8 ±0.1	4.8 ±0.1	4.7 ±0.1	6.4 ±0.1	7.6 ±0.1
VS	%	4.0 ±0.1	4.0 ±0.1	4.0 ±0.1	4.0 ±0.1	4.0 ±0.1	5.4 ±0.1	6.4 ±0.1
SCOD	g l^{-1}	11.5 ±1.5	10.8 ±1.3	10.1 ±1.5	9.4 ±1.7	8.7 ±2.1	11.8 ±1.7	14.0 ±0.9
NH_4-N	g l^{-1}	0.8 ±0.1	0.7 ±0.1	0.6 ±0.1	0.6 ±0.1	0.5 ±0.1	0.7 ±0.1	0.8 ±0.1
N_{tot}	g l^{-1}	2.1 ±0.2	1.9 ±0.1	1.7 ±0.1	1.6 ±0.0	1.4 ±0.3	1.9 ±0.1	2.3 ±0.1
Digestate								
TS	%	4.4 ±0.1	3.9 ±0.2	3.1 ±0.4	3.5 ±0.2	3.3 ±0.3	4.0 ±0.1	4.8 ±0.2
VS	%	3.2 ±0.1	2.9 ±0.2	2.3 ±0.3	2.7 ±0.2	2.5 ±0.3	3.2 ±0.1	3.9 ±0.2
SCOD	g l^{-1}	10.7 ±3.4	7.7 ±0.5	7.0 ±1.3	6.4 ±0.7	5.0 ±0.6	6.5 ±0.6	7.7 ±1.0
NH_4-N	g l^{-1}	1.0 ±0.1	0.9 ±0.1	0.7 ±0.0	0.5 ±0.0	0.5 ±0.0	0.6 ±0.0	0.8 ±0.0
N_{tot}	g l^{-1}	2.3 ±0.2	2.1 ±0.1	1.5 ±0.1	1.7 ±0.1	1.6 ±0.1	1.7 ±0.2	2.0 ±0.0
pH		7.6 ±0.1	7.5 ±0.1	7.5 ±0.1	7.5 ±0.1	7.3 ±0.1	7.4 ±0.1	7.6 ±0.1
TS removal [1]	%	12	20	36	27	31	37	37
VS removal [1]	%	20	27	43	33	38	40	40
CH_4 conc.	%	49 ±3	49 ±2	51 ±2	51 ±1	51 ±1	53 ±2	52 ±3
Specific CH_4 yield	m^3 kg^{-1} VS_{added}	0.151 ±0.044	0.145 ±0.009	0.159 ±0.019	0.213 ±0.017	0.188 ±0.019	0.184 ±0.023	0.157 ±0.028
	m^3 t^{-1} ww	6.0 ±1.7	5.8 ±0.4	6.4 ±0.8	8.5 ±0.7	7.5 ±0.8	9.9 ±1.2	10.1 ±1.8
% of total CH_4 potential in substrates [1]		65	63	70	95	85	83	71
% of short-term CH_4 potential in substrates [1]		74	73	83	116	106	104	88

[1] Calculated on basis of average values

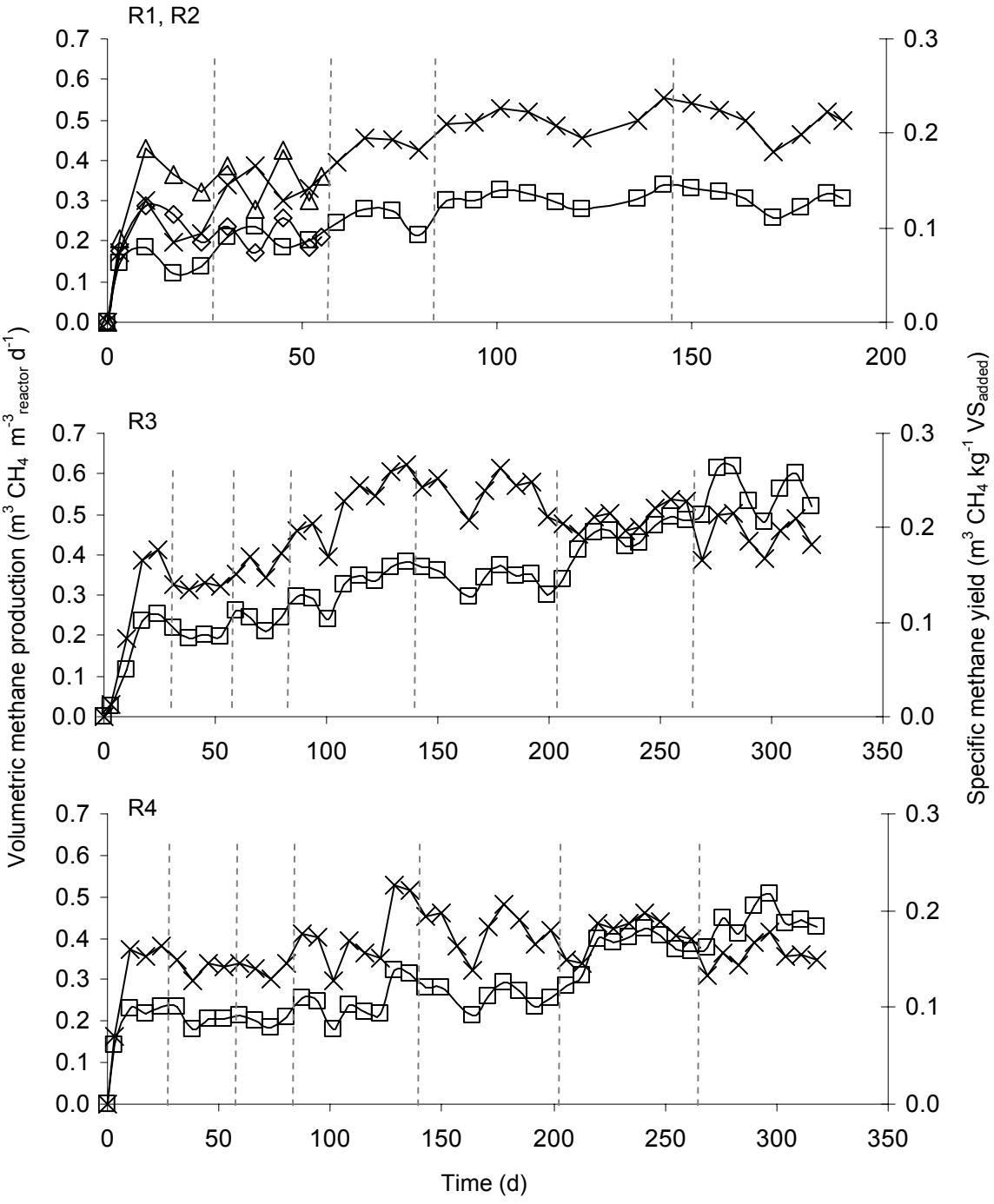

FIGURE 8 Volumetric methane production and specific methane yields as weekly averages in digestion of manure alone (R1) and in co-digestion of cow manure with sugar beet tops (R2), grass (R3) and straw (R4). Dashed lines represent the changes in feeding mode in R2, R3 and R4. Note the different time scale in R1, R2 compared with R3 and R4. ◊ Volumetric CH_4 production in R1; Δ Specific CH_4 yield in R1; □ Volumetric CH_4 production in R2, R3 and R4; x Specific CH_4 yield in R2, R3 and R4.

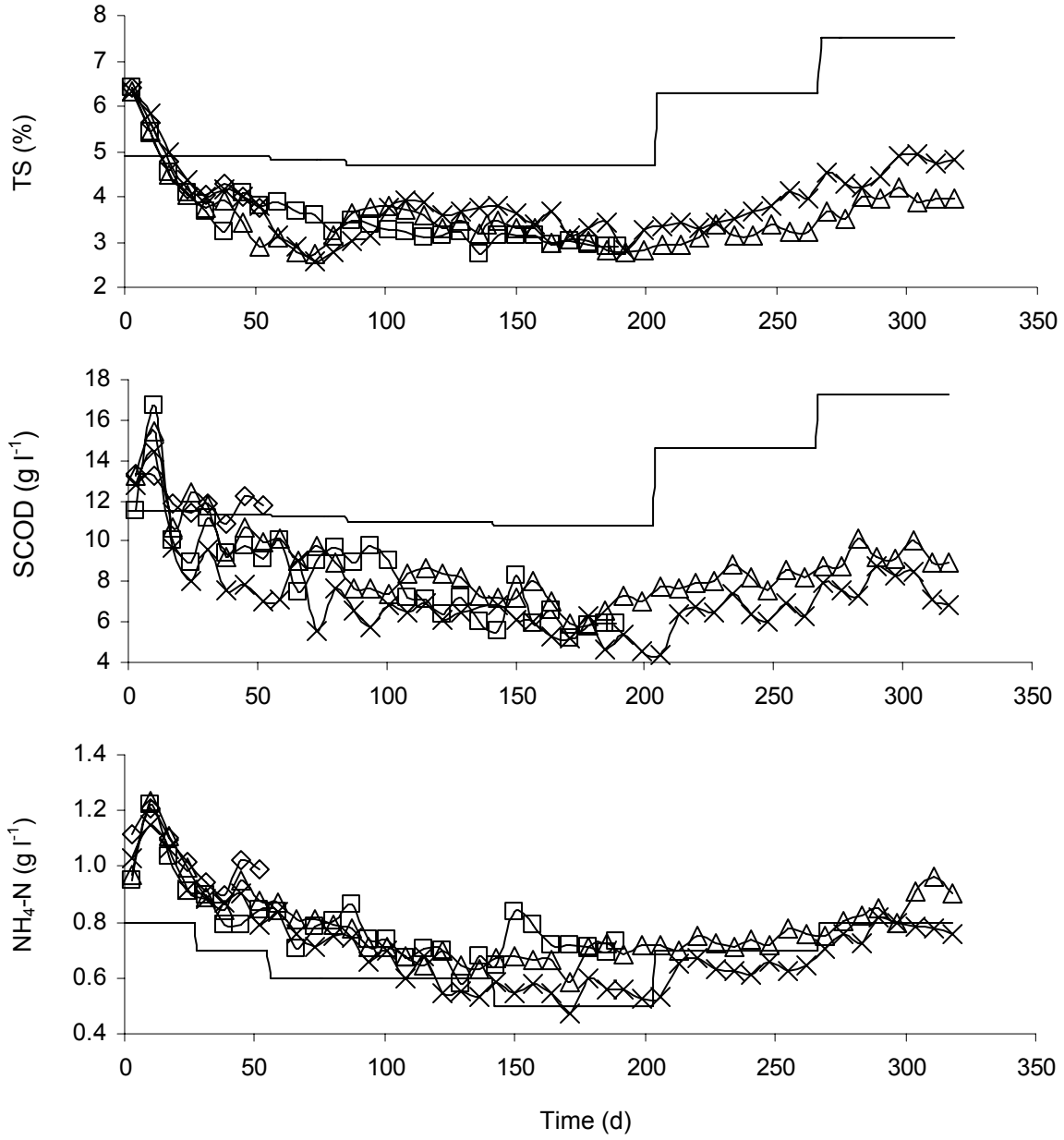

FIGURE 9 Characteristics of digestates from CSTRs as weekly averages. The solid lines represent the concentrations in the feedstock to reactor R3 as an example. ◊ R1; □ R2; Δ R3; x R4.

TABLE 21 Post-methanation potentials of digestates from co-digestion of cow manure with sugar beet tops (R2), grass (R3) and straw (R4) at different temperatures (average values of replicates ± standard deviations).

Sampling day	T	Post-methanation potential					
		R2		R3		R4	
	(° C)	(m^3 CH_4 kg^{-1} VS_{added})	(m^3 CH_4 t^{-1} ww)	(m^3 CH_4 kg^{-1} VS_{added})	(m^3 CH_4 t^{-1} ww)	(m^3 CH_4 kg^{-1} VS_{added})	(m^3 CH_4 t^{-1} ww)
140	5	0.002 ±0.000	0.1 ±0.0	0.002 ±0.000	0.0 ±0.0	0.006 ±0.001	0.2 ±0.0
	20	0.076 ±0.001	1.7 ±0.0	0.073 ±0.007	1.8 ±0.2	0.073 ±0.008	2.0 ±0.2
	35	0.142 ±0.001	3.1 ±0.0	0.148 ±0.004	3.5 ±0.1	0.168 ±0.005	4.5 ±0.1
190	5	0.002 ±0.000	0.0 ±0.0	0.001 ±0.000	0.0 ±0.0	0.002 ±0.000	0.1 ±0.0
	20	0.081 ±0.003	1.7 ±0.1	0.080 ±0.001	1.8 ±0.0	0.082 ±0.003	2.1 ±0.1
	35	0.140 ±.0.005	2.9 ±0.1	0.133 ±.0009	2.9 ±0.2	0.151 ±0.009	3.8 ±0.2
265	5	-	-	0.003 ±0.001	0.1 ±0.0	0.006 ±0.001	0.2 ±0.0
	20	-	-	0.078 ±0.001	2.0 ±0.0	0.091 ±0.005	2.9 ±0.2
	35	-	-	0.142 ±0.003	3.7 ±0.1	0.162 ±0.003	5.2 ±0.1
318	5	-	-	0.003 ±0.000	0.1 ±0.0	0.009 ±0.000	0.4 ±0.0
	20	-	-	0.103 ±0.004	3.2 ±0.1	0.120 ±0.005	4.7 ±0.2
	35	-	-	0.182 ±0.007	5.6 ±0.2	0.197 ±0.007	7.7 ±0.3

4.4 Anaerobic digestion of energy crops in batch leach bed processes

4.4.1 One-stage leach bed reactors (Run 1)

The one-stage leach bed reactor (LB1) was operated with the clover-free grass silage as substrate, inoculated with digested sludge from a mesophilic farm digester (Table 10–11), and run with internal recirculation of leachate with pH adjusted to 7 before being returned to the reactor (IV). pH of the effluent initially decreased to 4.8 on day 1, but increased quickly, reaching 6.2 by day 3 and 7.0 by day 9, and thereafter varying between 6.9 and 7.8 for the rest of the run (Fig. 10). SCOD in effluent from LB1 reached a level of 15 g l^{-1} after 24 h of leachate recirculation, after which it started to decrease, dropping to 9 g l^{-1} by day 9, but then peaking again at 15 g l^{-1} on day 13 (Fig. 10). After that the SCOD decreased steadily, reaching a concentration of 2 g l^{-1} by day 55. VFA concentrations followed a different pattern, peaking at 5.2 g l^{-1} (total VFA, TVFA) on day 13, corresponding to 7.3 g COD l^{-1} and 52% of SCOD, and decreasing steadily from then on to < 1 g l^{-1} (TVFA). The VFAs present in the

highest concentrations were acetate (up to 2.4 g l^{-1}), propionate (up to 1.1 g l^{-1}) and butyrate (up to 1.2 g l^{-1}) (Fig. 10).

Methane production remained low until day 9 (< 5 ml d^{-1}), then started to increase and peaked at 123 ml d^{-1} on day 20 (Fig. 11). Methane concentration in the gas from LB1 remained below 2% until day 9, then started to increase and reached 36% by day 16. From then on, the methane concentration varied between 34 and 53% (Fig. 11). Carbon dioxide was produced from the beginning, the production peaking at 356 ml d^{-1} on day 13 (Fig. 11). The total specific methane yield in run 1 was 0.060 m^3 CH_4 kg^{-1} VS_{added} and 15 m^3 CH_4 t^{-1} ww after 55 days of operation, corresponding to 20% of the total and 23% of the short-term (30 d) methane potential in grass silage (Table 22). After 30 d of operation, the specific methane yield in run 1 was 0.033 m^3 CH_4 kg^{-1} VS_{added}, corresponding to 11% of the total and 13% of the short-term (30 d) methane potential in grass silage. The total VS removal in run 1 amounted to 34% (Table 22). The post-methanation potential of the digestate from run 1 was 0.204 m^3 CH_4 kg^{-1} digestate VS_{added}, corresponding to 21 m^3 CH_4 t^{-1} ww of digestate, and 93% of total methane production (methane production in reactor and in post-methanation) (Table 23).

4.4.2 Two-stage process: leach bed reactor and UASB

4.4.2.1 Effect of pH adjustment (Runs 2 and 3)

In runs 2 and 3, leach bed reactors LB2 and LB3, fed with the clover-free grass silage, were operated in conjunction with a UASB, with the leachate collected at the bottom of leach bed reactors circulated to UASB, and the UASB effluent being returned at the top of the leach bed reactors (IV). In run 3, the pH of the influent to the leach bed reactor (LB3) was adjusted to 6. In runs 2 and 3, SCOD in effluents from LB2 and LB3 reached a level of 11–12 g l^{-1} after 24 h of leachate recirculation, and circulation to the UASB was initiated. Circulation to the UASB was continued until day 9 in run 2 and until day 10 in run 3, when the SCOD concentration in the effluent from LB2 and LB3 had dropped to below 1 g l^{-1}. At this stage, the pH of the effluents from LB2 and LB3 were 7.3 and 5.6, respectively (Fig. 10). After the UASB was disconnected, the pH in the effluent from LB2 initially decreased to 6.1, and remained slightly below 7 for the rest of the run, while SCOD in the effluent from LB2 increased, peaking at 3.5 g l^{-1} on day 23, thereafter decreasing slowly to 1.8 g l^{-1} at the end of the run. In the effluent from LB3, SCOD remained between 1.5 and 1.8 g l^{-1} until the end of the run, pH varying between 5.5 and 5.8. In the effluent from LB2, TVFA and acetate peaked at 1.6 g COD l^{-1} and 1.0 g l^{-1}, respectively, on day 13, thereafter decreasing steadily to < 1 g COD l^{-1} (TVFA) by day 34, whereas the concentration of propionate was highest (0.3 g l^{-1}) on day 34 and butyrate was present in lower amounts (about 0.1 g l^{-1}) (Fig. 10). The VFAs present in the leachate from LB3 before day 10 were acetate, propionate and butyrate, in concentrations 0.1–0.7 g l^{-1}, TVFA peaking at 1.8 g COD l^{-1} on day 7. However, after the UASB was disconnected, propionate, in concentrations of 0.1–0.2 g l^{-1}, was the only VFA present (Fig. 10). In run 2, the proportion of TVFA of SCOD

was highest, 75%, on day 13, whereas in run 3, the corresponding figure was 42% on day 7.

In run 2, the COD reduction in the UASB was initially 96% and decreased to 56% on day 9 as the SCOD concentration in the influent fell to 1 g l^{-1}, whereas in run 3 the COD reduction showed greater initial fluctuation (COD reduction 79–93%), thereafter decreasing to 45% by day 10. Methane concentrations in the gas produced in the UASB varied between 60–72% in run 2 and 46–60% in run 3, and the daily CH_4 production from the UASB peaked at 1 410 ml d^{-1} on day 6 in run 2 and at 891 ml d^{-1} on day 4 in run 3 (Fig. 11). During run 2, VFAs were not present in the UASB effluents, but during run 3, acetate and propionate were present in the UASB effluent in concentrations up to 0.3 g l^{-1} (data not shown).

Methane production in LB2 and LB3 remained low until day 7, then started to increase, peaking at 56 ml d^{-1} on day 21 and at 60 ml d^{-1} on day 35 in run 2, and at 63 ml d^{-1} on day 17 in run 3. Methane concentration in the gas from LB2 remained below 1% until day 6, then started to increase, peaking at 47% on day 34, whereas in LB3 methane concentration remained below 3% until day 7, thereafter increasing for the rest of the run, finally ending at 43% on day 31. In LB2, carbon dioxide production peaked at 365 ml d^{-1} on day 1 and at 316 ml d^{-1} on day 9, thereafter steadily decreasing, and varying between 60–110 ml d^{-1} for the rest of the run, whereas in LB3 carbon dioxide production peaked at 167 ml d^{-1} on day 1, at 284 ml d^{-1} on day 3 and at 210 ml d^{-1} on day 10 (Figure 11). The total specific methane yields were 0.197 m^3 CH_4 kg^{-1} VS_{added} and 47 m^3 CH_4 t^{-1} ww after 55 days of operation in run 2, and 0.103 m^3 CH_4 kg^{-1} VS_{added} and 25 m^3 CH_4 t^{-1} ww after 31 days of operation in run 3. Of this methane yield, 80 and 76% in runs 2 and 3, respectively, originated from the UASB. After 30 d of operation, the specific methane yields were 0.177 and 0.102 m^3 CH_4 kg^{-1} VS_{added} in runs 2 and 3, respectively. Total VS removal amounted to 55% in run 2 and 39% in run 3 (Table 22). The post-methanation potentials of the digestates were 0.141 m^3 CH_4 kg^{-1} digestate VS_{added} in run 2 and 0.160 m^3 CH_4 kg^{-1} digestate VS_{added} in run 3 (Table 23).

4.4.2.2 Characterisation of the residues (Run 4)

Six leach bed reactors installed in parallel and connected to a common UASB were operated with the clover-free grass silage as substrate in run 4, and the leach bed reactors were terminated sequentially during the run in order to chemically characterise the residues and evaluate the removal of different fractions of organic matter in various stages of digestion (IV). The SCOD in the effluent from the leach bed reactors reached a level of 37 g l^{-1} after 24 h of leachate recirculation, and circulation to the UASB was initiated. Circulation to the UASB was continued until day 17, when the SCOD in the effluent from leach bed reactors had dropped to below 2 g l^{-1} and the pH of the effluent was 7.5 (Fig. 10). After the UASB was disconnected, the SCOD in the effluent from the leach bed reactors slightly increased, peaking at 3.3 g l^{-1} on day 20, thereafter varying between 1.5 and 2.6 g l^{-1} until the end of the run. VFA concentrations followed a pattern very similar to that of COD, acetate and propionate peaking at 1.8 and 0.5 g l^{-1}, respectively, on day 3, TVFA corresponding to 2.8 g COD l^{-1}

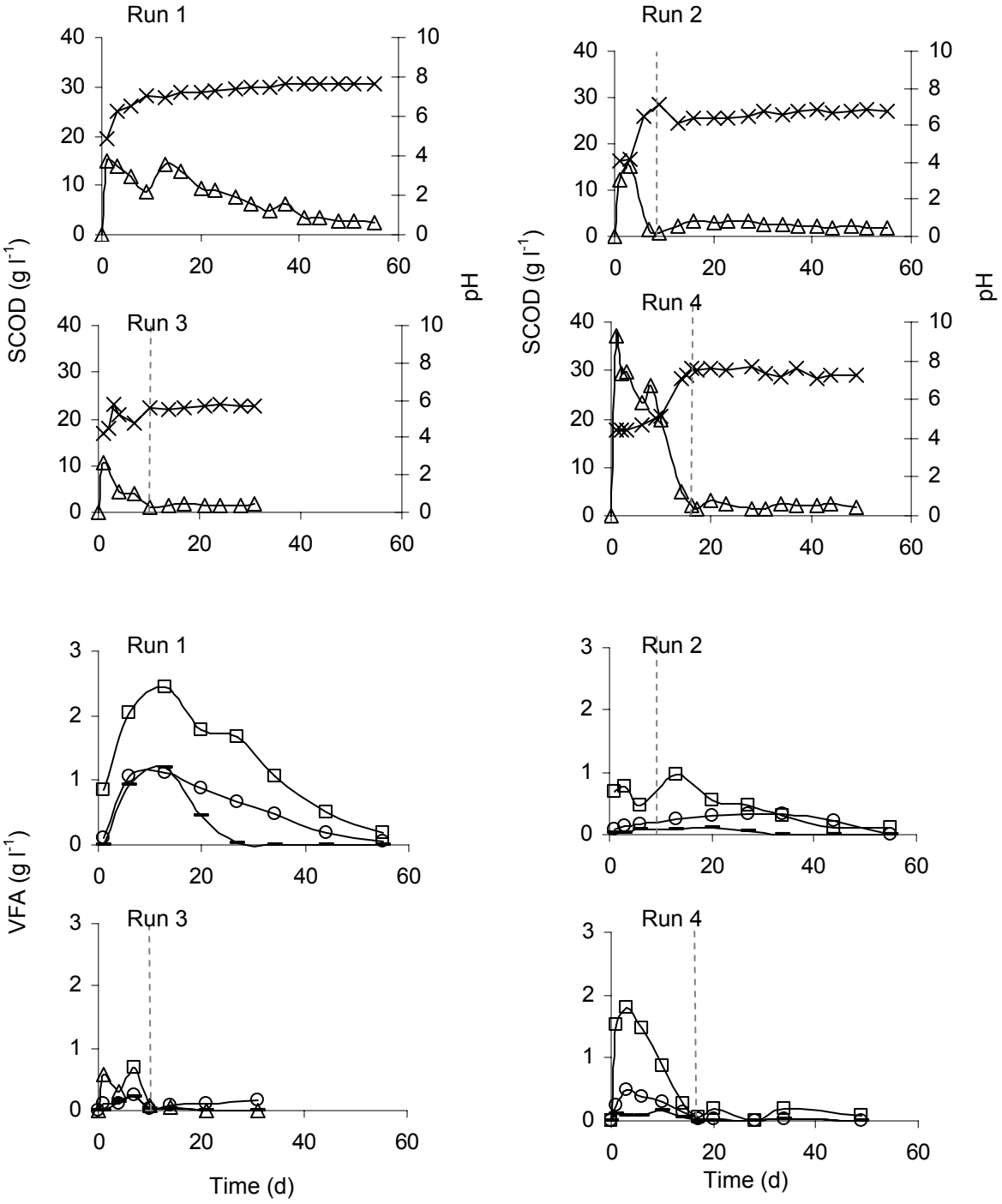

FIGURE 10 SCOD and VFA concentrations and pH in effluent from the leach bed reactors in the one-stage leach bed process (run 1) and in the leach bed – UASB processes, without (runs 2 and 4) and with (run 3) pH adjustment. Dashed lines mark the time when the leach bed reactors were disconnected from the UASB. Δ SCOD; x pH; □ Acetate; ○ Propionate; – Butyrate.

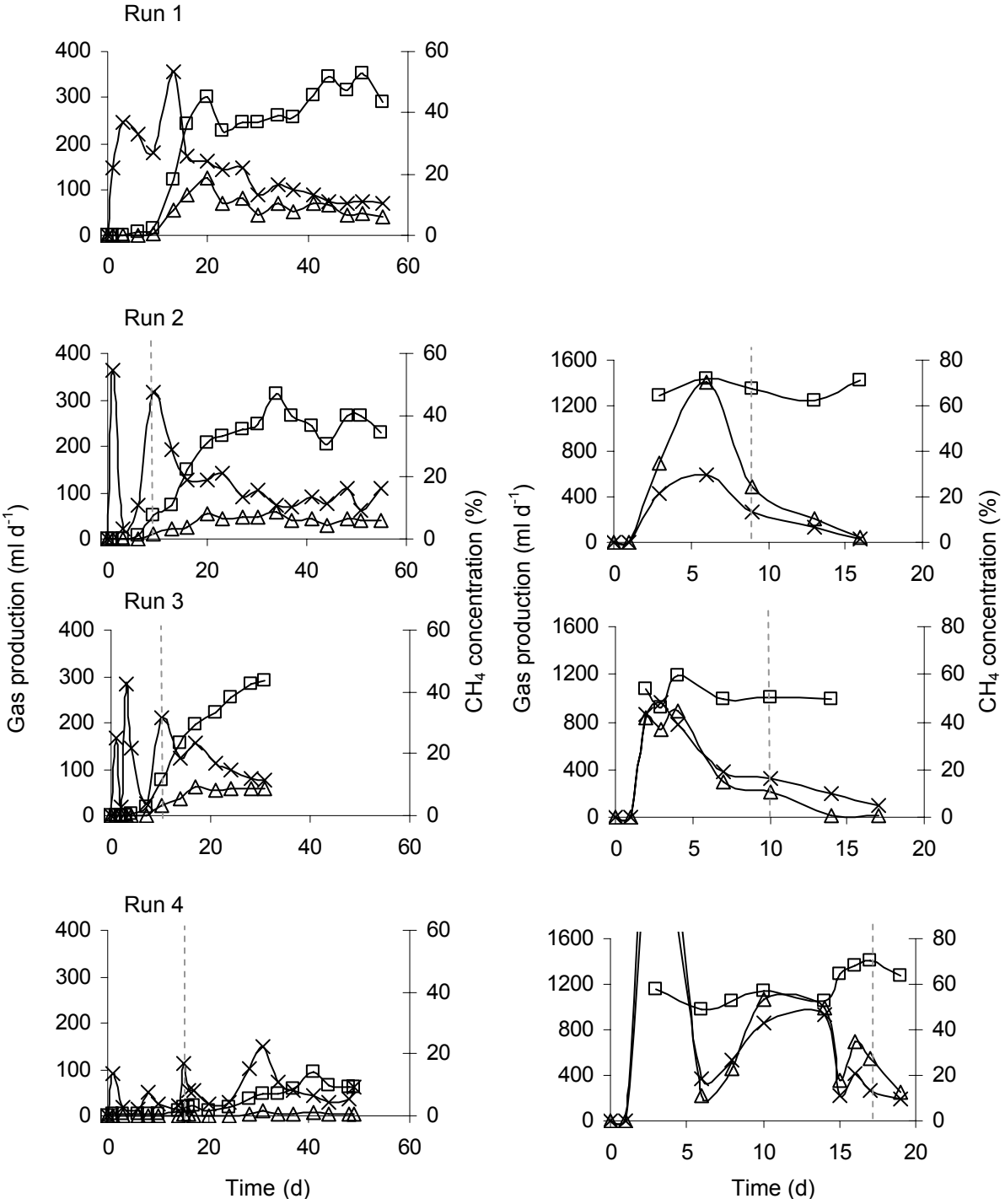

FIGURE 11 Daily gas production and methane concentrations in the one-stage leach bed process (run 1) and in the leach bed – UASB processes, without (runs 2 and 4) and with (run 3) pH adjustment (left: leach bed reactors, right: UASB). Dashed lines mark the time when the leach bed reactors were disconnected from the UASB. In run 4, values for gas production in UASB on day 3 are out of scale (3 466 ml d^{-1} CH_4 and 2 683 ml d^{-1} CO_2, respectively). Δ CH_4 production; x CO_2 production; □ CH_4 concentration.

TABLE 22 Specific methane yields and total VS removals in the one-stage leach bed process (run 1) and in the leach bed – UASB processes, without (runs 2 and 4) and with (run 3) pH adjustment.

Run		1	2	3	4
Total specific methane yield	(m^3 CH_4 kg^{-1} VS_{added})	0.060	0.197	0.103	0.107
	(m^3 CH_4 t^{-1} ww)	15	47	25	26
	(% of total methane potential)	20	66	34	36
	(% of short-term methane potential)	23	75	39	41
Short-term specific methane yield [a]	(m^3 CH_4 kg^{-1} VS_{added})	0.033	0.177	0.102	0.105
	(m^3 CH_4 t^{-1} ww)	8	42	25	25
	(% of total specific methane yield)	55	89	99	99
	(% of total methane potential)	11	59	34	35
	(% of short-term methane potential)	13	67	39	40
Total VS removal	(%)	34	55	39	42

[a] Methane yield after 30 d

TABLE 23 Post-methanation potentials, determined at 35 °C, of digestates from the one-stage leach bed process (run 1) and from the leach bed – UASB processes, without (run 2) and with (run 3) pH adjustment (average values of replicates ± standard deviations, where applicable).

Run	Post-methanation potential		
	(m^3 CH_4 kg^{-1} VS_{added})	(m^3 CH_4 t^{-1} ww)	(% of total methane production) [a]
1	0.204 ±0.013	21 ±1	93
2	0.141 ±0.025	22 ±4	42
3	0.160 ±0.012	19 ±1	52

[a] Methane production in reactor(s) and in post-methanation.

and decreasing steadily from then on to < 1 g COD l^{-1} by day 14. Butyrate was present in low amounts (about 0.1 g l^{-1}). After the UASB was disconnected, the pH in the leach bed effluents varied between 7.1 and 7.7 for the rest of the run (Fig. 10).

The COD reduction in the UASB varied between 96 and 99% until day 10 and then decreased to 47–49% as the SCOD concentration in the leachate declined. Methane concentrations in the gas produced in the UASB varied between 49 and 70%, and the daily CH_4 production from UASB peaked on day 3 at 3 466 ml d^{-1}. Decreases in gas production in UASB on days 6 and 14 were due to the pumps having stopped during the night. Methane production in the leach bed reactors remained low throughout the run, reaching a maximum of 10 ml d^{-1} on day 31. Methane concentration in the gas from the leach bed reactors remained below 1% until day 10, then started to increase slowly, reaching 14% on day 41. Carbon dioxide production peaked several times, at 91 ml d^{-1} on day 1, at 112 ml d^{-1} on day 15, and at 149 ml d^{-1} on day 31, thereafter steadily decreasing (Fig. 11). The total specific methane yield in run 4 was 0.107 m^3 CH_4 kg^{-1} VS_{added} and 26 m^3 CH_4 t^{-1} ww after 49 days of operation (Table 22), corresponding to 36% of the total and 41% of the short-term (30 d) methane potential in grass silage. Of this methane yield, 98% originated from the UASB, and 2% from the leach bed reactors. The specific methane yield in run 4 after 30 d of operation was 0.105 m^3 CH_4 kg^{-1} VS_{added} (Table 22).

The extent of VS removal was determined each time a reactor was terminated. After 1 day of leachate recirculation, VS removal had reached 16% (Fig. 12). After day 1, the reduction in VS slowed down, reaching 30% by the time methanogenesis had begun in the leach bed reactors (day 17). Total VS removal in run 4 amounted to 42%. The reduction in heat content correlated well with the VS removals, reaching 45% by the end of the run (Fig. 12).

The composition of grass was analysed on day 0 and after 1, 10 and 49 days of digestion. 17% of grass TS initially consisted of lignin (Klason lignin and acid-soluble lignin), 45% of carbohydrates, 8% of extractives and 10% of proteins (Fig. 12). After 1 day of digestion, 11% of Klason lignin and 24% of acid soluble lignin had been removed from the solid residue, whereas proteins, extractives and carbohydrates had degraded by 34, 12 and 10% (Fig. 12). After 10 days of digestion extractives were the most rapidly removed component, their removal reaching 59%. At the end of digestion (after 49 d), 74, 51 and 39% of extractives, proteins and carbohydrates, respectively, had been removed from the solid residue, whereas the removal of Klason lignin and acid soluble lignin amounted to 17 and 58%, respectively (Fig. 12). The residue after completion of digestion consisted of 23% (from TS) of lignin, 50% of carbohydrates, 4% of extractives and 9% of proteins.

4.4.3 Two-stage process: leach bed reactor and methanogenic filter

Energy crops (willow in run A, sugar beet in run B and grass-clover silage in run C) were digested in pilot leach bed – MF processes (Table 12, Fig. 13, V). In run A with willow, the COD in leachate from 1st stage reached a level of 12 g l^{-1} on day 3 (Fig. 13), and circulation over the MFs could be initiated. The loading rate to the MFs was maintained at 10 kg COD m^{-3} d^{-1} for the first 3 days, after which it decreased with decreasing COD concentration, until circulation over MFs was terminated on day 9. By then the methane concentrations in gas produced in the 1st stages were 47 and 43% in H1 and H2, respectively. The reactors were terminated after 82 d of operation, after which 84% of total CH_4 was produced in the 1st stages, and, due to low solubilisation of organic matter, only 16% in the MFs.

In run B with sugar beet, COD values of 42–44 g l^{-1} were observed in the leachates from 1st stages after 24 h of internal circulation (Fig. 13), and circulation over the MFs was initiated at loading rate of 10 kg COD m^{-3} d^{-1}. However, this loading led to a drop in pH in MFs (pH 5.7 and 6.7 in MF1 and 2, respectively, on day 3), and on day 3 the MFs were put on internal circulation. MF2 recovered rapidly (pH 7.2 on day 4), and loading rates of 8–19 kg COD m^{-3} d^{-1} were applied between days 7–23. By day 23 the CH_4 concentration in gas from H2 had increased to 56%, pH was 7.3, and circulation over MF2 was terminated. It took a longer time for MF1 to recover from the pH drop (pH < 7 until day 14), and a low loading rate of 3 kg COD m^{-3} d^{-1} was applied on day 14. Loading rate was maintained at 5–10 kg COD m^{-3} d^{-1} until day 44, when MF1 was considered redundant, pH in H1 being 7.5 and CH_4 concentration 68%. Run B was terminated on day 55. In this run, 17% of total CH_4 was produced in the 1st stages, and 83% in the MFs.

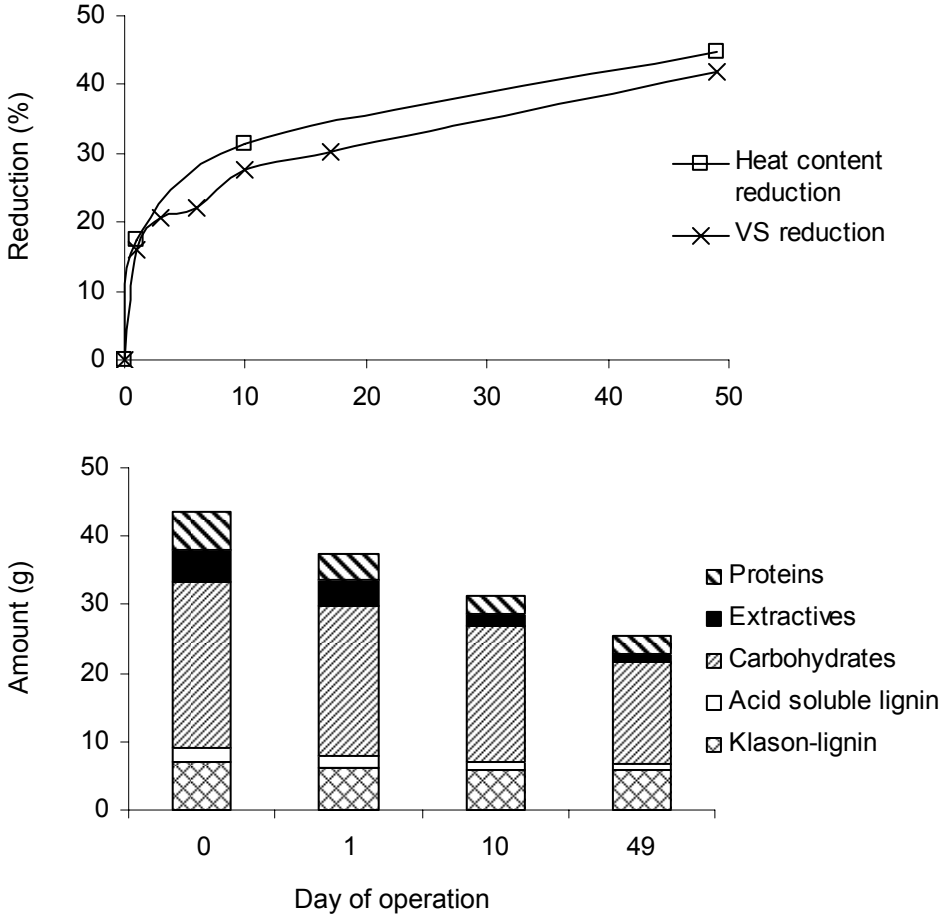

FIGURE 12 Reduction in VS and heat content, and amounts of analysed fractions in untreated grass silage (day 0) and in residues after different periods of digestion in the leach bed – UASB process (run 4).

In run C with grass-clover silage, COD values of 55–61 g l^{-1} were observed in the leachate from the 1st stage after 24 h of internal circulation (Fig. 13), and circulation over the MFs was started at an OLR of 5 kg COD m^{-3} d^{-1}. Loading was then gradually increased to 10 and 20 kg COD m^{-3} d^{-1}. With these loading rates MF2 showed reliable performance with little fluctuations in pH (7.6–7.9), whereas MF1 had low COD removal efficiency and larger fluctuations in pH (7.1–7.8). H2 became methanogenic slightly faster, and MF2 was closed on day 22, whereas circulation was continued over MF1 until day 28. Run C was terminated on day 50. In run C, 36% of total CH_4 was produced in the 1st stages and 64% in the MFs. Despite the pH problems in MF1 in runs B and C, the total methane yields of the two reactor set-ups were very similar, and average values are presented in Table 24. In pilot reactors, specific methane yields of 0.162, 0.382 and 0.390 m^3 CH_4 kg^{-1} VS_{added} were obtained in run A with willow, run B with sugar beet and run C with grass-clover silage, respectively (Table 24). In digestion of willow, 59% of the total methane yield was attained on day 30, whereas the corresponding figures in digestion of sugar beet and grass-clover silage were 84 and 85%, respectively (Fig. 14). The VS reductions in digestion of willow, sugar beet and grass-clover silage amounted to 46, 96 and 59%, respectively (Table 24).

Organically bound nitrogen (org-N) and mineralised nitrogen (NH_4-N) were analysed in all solid and liquid fractions in the start and end of the pilot experiments (Fig. 15). In the end of run A with willow, 18% of the total nitrogen in the substrate had been converted to NH_4-N, which was equally distributed between the liquid and solid phases. In digestion of sugar beet (run B), 88% of total nitrogen in the substrate was converted to NH_4-N, and 98% of the NH_4-N was in the liquid phase at the end of digestion. In digestion of grass-clover silage (run C), the amount of nitrogen in the substrate was the highest (23 kg), but the mineralisation rate was lower (40% N_{tot}), and at the end of the run, 29% of the nitrogen was present as NH_4-N in the liquid phase (Fig. 15). Nitrogen concentrations as related to TS in the solid fractions increased two-fold in digestion of willow and grass-clover silage (runs A and C) and four-fold in digestion of sugar beet (run B). Solid residues from digestion of sugar beet and grass-clover silage had C/N ratios of 10–11 and 7–9, respectively, whereas that from digestion of willow was 24–31.

Concentrations of heavy metals in different fractions during and after digestion were determined, and metal concentrations in the digestates were compared with the limit values for use of digestate as fertiliser in Sweden (Swedish EPA 2005) (Table 25). In the solid residues from digestion of grass-clover silage and sugar beets metal concentrations were below the limit values, but in the solid residues from digestion of willow cadmium concentration (4.7 µg g^{-1} TS) exceeded the limit value for agricultural use of digestates (2.0 µg g^{-1} TS) (Table 25). In runs with sugar beet and grass–clover silage, metals known to be abundant in agricultural soils (Pb, Cd, Ni, Cu, Zn; Swedish EPA 2005) were monitored in the liquid in the 1st stage both during the acidic phase (day 3 in run B and day 2 in run C) and at the end of each run. With few exceptions these heavy metals were strongly mobilised during the acidic phase with the concentrations in the leachate being up to 24 fold higher than during the neutral pH conditions prevailing at the end of the runs (Fig. 16).

TABLE 24 Specific methane yields and removal of different carbon fractions and of heat content in pilot experiments with leach bed – MF processes.

Run		A	B	C
Substrate		Willow	Sugar beet	Grass silage
Specific methane yield	(m^3 CH_4 kg^{-1} VS_{added})	0.162	0.382	0.390
"	(m^3 CH_4 kg^{-1} TS_{added})	0.159	0.339	0.342
"	(m^3 CH_4 t^{-1} ww)	79	68	109
VS removal	(%)	46	96	59
Heat content removal	(%)	35	94	51
Crude fibre removal	(%)	32	87	43
NDF removal	(%)	32	88	27
ADF removal	(%)	49	83	41

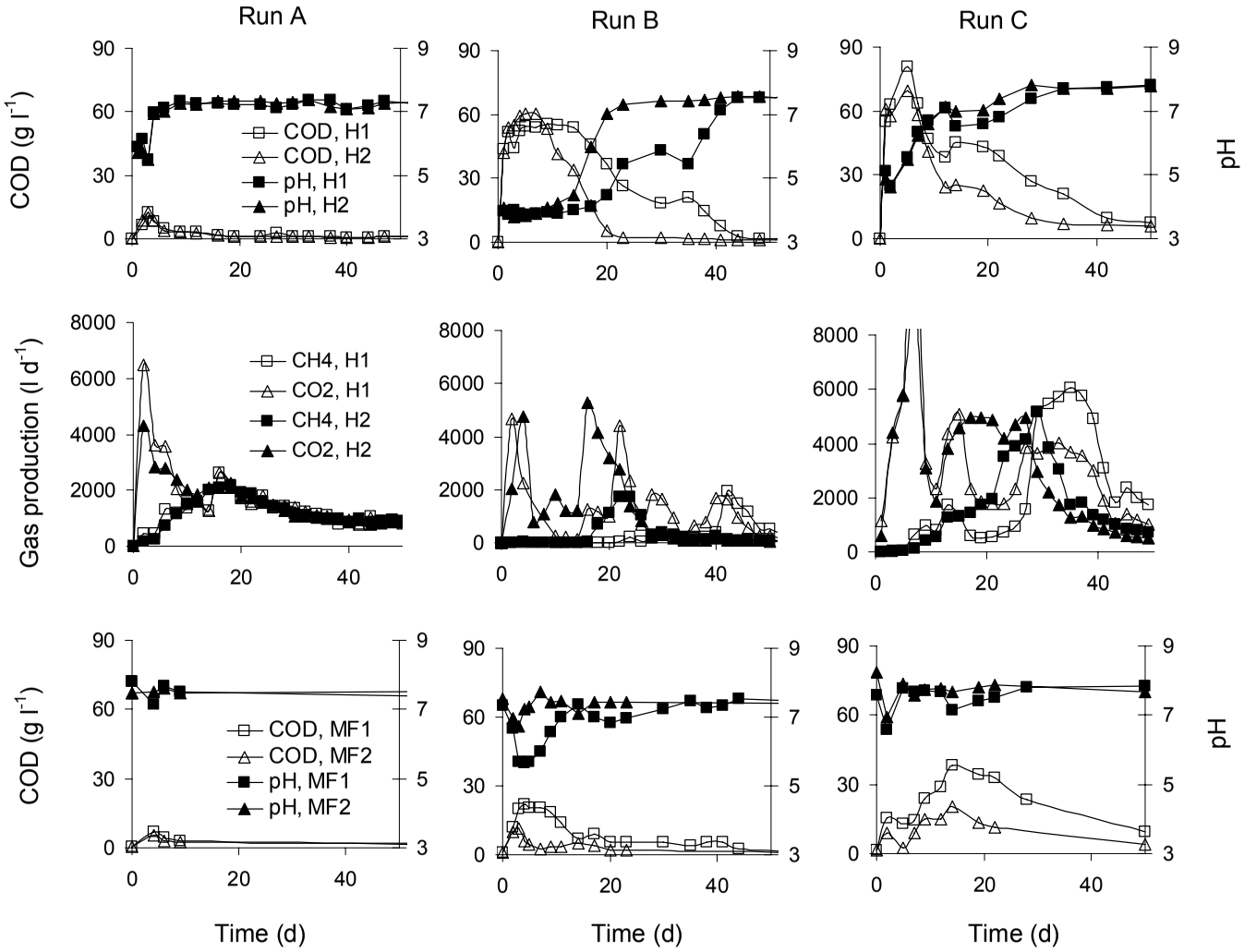

FIGURE 13 pH and COD profiles of effluents from all reactors and daily gas productions in 1st stage in pilot experiments with leach bed – MF processes. Values for CO_2 production in run 3 for day 6 (out of scale) are 21 517 (H1) and 12 479 (H2) l d^{-1}.

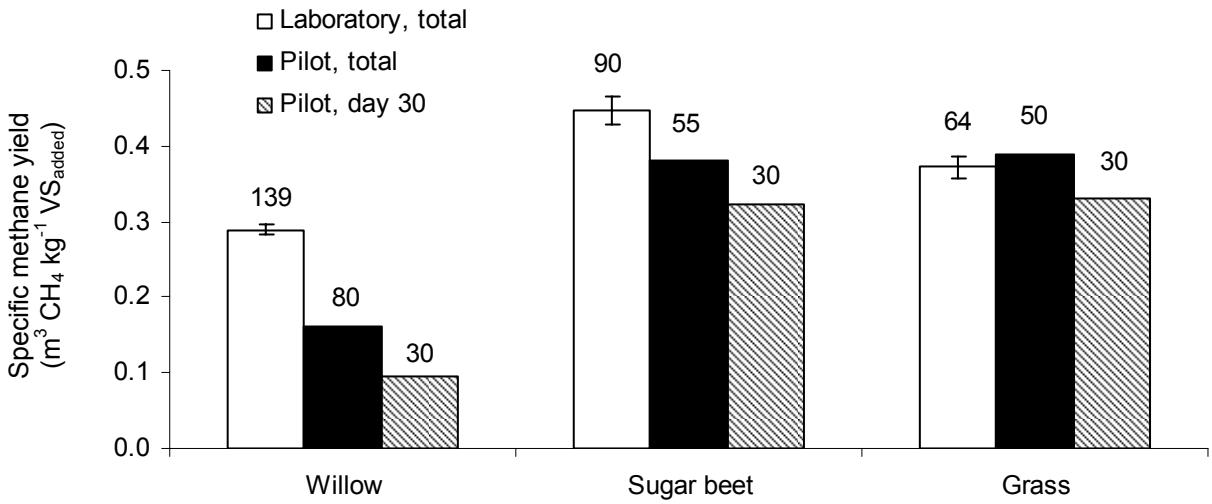

FIGURE 14 Total and short-term (30 d) specific methane yields of different substrates in laboratory batch assays and pilot experiments with leach bed – MF processes. For pilot experiments with sugar beet, values from set-up 1 by day 30 are excluded due to operational problems. Values above bars represent the duration of the experiment in days. Error bars indicate the standard deviation between replicates, where applicable.

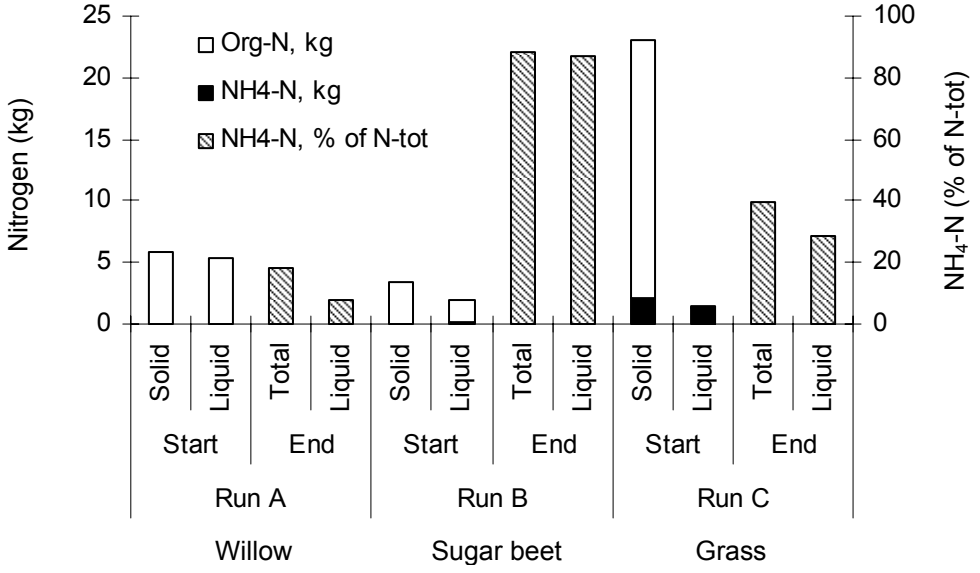

FIGURE 15 Initial distribution of organic (org-N) and mineralised nitrogen (NH_4-N) in substrate and process liquid, and percentage of mineralised nitrogen after digestion as total and in liquid phase in pilot experiments with leach bed – MF processes.

TABLE 25 Concentrations of heavy metals in the solid residues from pilot experiments with leach bed – MF processes, and limit values for utilisation of digestate as soil fertiliser (Swedish EPA 2005).

Metals (µg g^{-1} TS)	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
Run (Substrate)								
A (Willow)	0.55	4.66	9.64	17.53	0.02	0.47	1.35	183.21
B (Sugar beet)	2.84	1.52	54.76	96.54	0.08	40.35	6.62	194.12
C (Grass)	1.23	0.56	23.56	58.82	0.05	9.90	4.05	107.23
Limit value	-	2	100	600	2.5	50	100	800

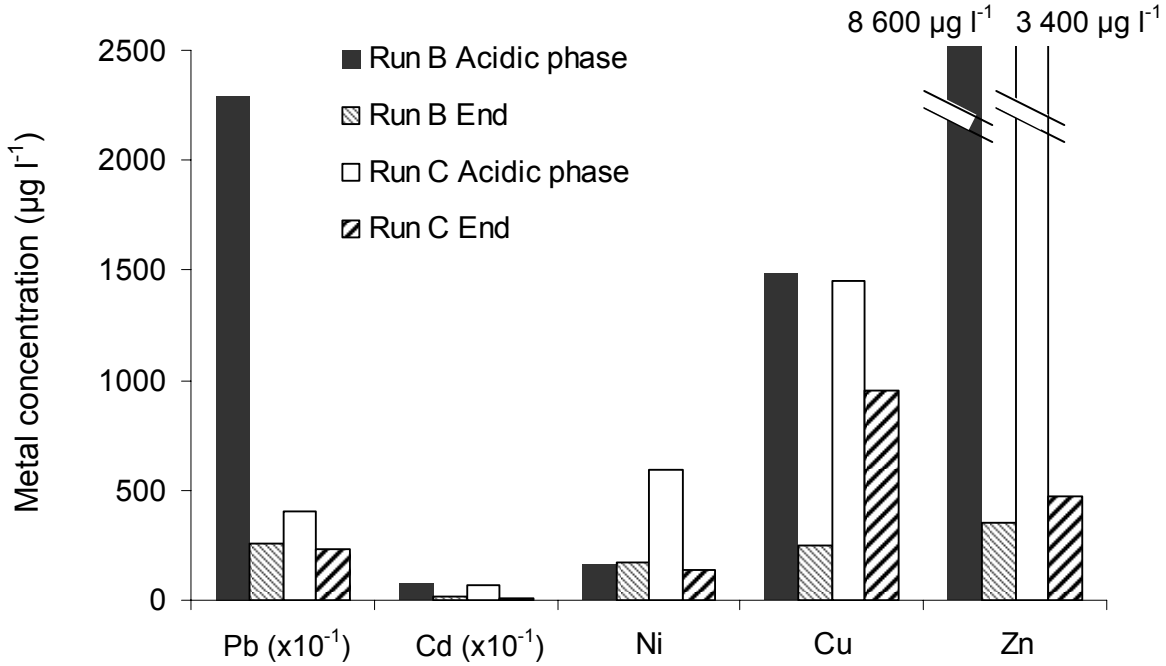

FIGURE 16 Metal concentrations in leachate from 1st stage under low pH (acidic phase) and neutral (end of run) conditions in pilot experiments with leach bed – MF processes. Values for zinc / acidic phase are out of range with 8 600 and 3 400 µg l^{-1} in runs B and C, respectively. Please note that concentrations of lead and cadmium are one order of magnitude lower.

5 DISCUSSION

5.1 Screening potential boreal energy crops and crop residues for methane production

The present results show the important effect that selection of crop species and harvest time can have on the obtainable methane production, as substantial differences were observed in methane potentials, both with different crops as well as with the same crop but with different harvest times, the methane and gross energy potentials from different crops varying from 400 to 5 400 m^3 CH_4 ha^{-1} and from 4 to 53 MWh ha^{-1} (I). In the present study, Jerusalem artichoke, reed canary grass and timothy-clover grass gave the highest methane potentials per hectare, corresponding to a gross energy potential of 28–53 MWh ha^{-1}. Thus, up to 3 000–5 000 m^3 of methane can potentially be obtained from one hectare of energy crops cultivated for biogas production, corresponding to approximately 40 000–60 000 kilometres in passenger car transport per hectare. Consequently, one to three passenger cars (average distance driven approximately 20 000 – 30 000 km per year) could potentially be fuelled by one hectare of energy crops. For example, if the area corresponding to the set aside agricultural land in Finland (195 929 ha in 2004, Statistics Finland 2005) was used for production of energy crop as substrates for biogas production, the methane production could potentially cover the yearly consumption of 8–25% of the passenger cars in Finland (2 346 726 passenger cars in 2004, Statistics Finland 2005).

In general, straws and grasses had high methane potentials per ww, whereas those of legumes were lower. The differences observed in the methane potentials of grass mixtures used in different experiments (I–V) were due to differences in the species composition of these mixtures, which has a large effect on the chemical characteristics as well as anaerobic degradability. It has been

previously reported that the methane potentials of grass mixtures are often higher than the methane potentials of individual grasses (Gunaseelan 1997). In the present study, grass mixtures containing clover (I, V) had significantly higher methane potentials than the mixtures without clover (II–IV), possibly due to the higher amounts of soluble nitrogen and lower amounts of lignin in the mixtures containing legumes with the ability to bind nitrogen from the atmosphere. The oat straw used in screening experiments (I) was drier (TS 90%) than the straw used in the co-digestion experiments (TS 64%) (III), which explains the lower methane yield per ww with the latter substrate.

The effect of harvest time on methane potentials per ww was remarkable with several crops, and the methane potentials mostly increased as the crops matured due to the decreasing water content, timothy-clover grass and rhubarb as the only exceptions. As the biomass yield with most crops also increases as the harvest is postponed, the later harvest seems to be more optimal in most cases. In the present study, the methane potentials per VS of timothy-clover grass and reed canary grass were higher at later harvest. In a previous study, harvest time had little effect on the methane potential of wheat, but rye grass harvested at flowering stage produced 50% more methane than when harvested at vegetative stage (Pouech et al. 1998), whereas with napier grass the methane potentials per VS were higher for the younger crop biomass, but the methane production rates were lower (Chynoweth et al. 1993). The patterns of methane production from legumes, Jerusalem artichoke and marrow kale were similar regardless of the maturity of the crop, and, thus, the later harvest is likely to be optimal with these crops due to the higher biomass yields obtained. The amount of non-structural carbohydrates, which are readily fermentable by micro-organisms, increased until mid-October in Jerusalem artichoke stands in Swedish growing conditions (Gunnarson et al. 1985), and the Jerusalem artichoke biomass can thus be harvested late in the growing season without jeopardising the anaerobic degradability.

The methane potential per VS was lowest for giant knotweed (1st harvest), whereas the methane potentials per ww were lowest for rhubarb (2nd harvest) and nettle (1st harvest). The methane potentials per hectare remained lowest for straws, lawn and sugar beet tops. Methane production from marrow kale proceeded slowly, as only 20% of total gas production was obtained within 30 d. The low methane potential of giant knotweed may be due to the high lignin content in this crop. Lignin is poorly degraded in anaerobic conditions, and the shielding effect of lignin due to the intense cross-linking of lignin with cellulose and hemicellulose severely limits the rate and extent of lignocellulose utilisation (Fan et al. 1981). The low methane potential per ww of rhubarb (2nd harvest) and sugar beet tops were due to their high water content. The methane potential per hectare for straws, lawn and sugar beet tops remained relatively low (400–1 500 m^3 CH_4 ha^{-1}) due to the apparent low biomass yields per hectare. However, one must take into account that these crops are produced as residues, by-products or wastes from normal agricultural practices, and the direct costs of production of these materials are often low. Low methane potentials could in some cases have been caused by inhibitory or toxic compounds contained in the crops. For example, lupine used in this study originated from natural stands of the variety *Lupinus polyphyllus.* Wild lupines are reported to contain high levels

of lupine alkaloids in their foliage and seeds, and these alkaloids are toxic or inhibitory to most organisms (Aniszewski 1993). However, alkaloid free cultivars have been developed (Langer & Hill 1982), and higher methane potentials may be obtainable by using these cultivars.

When estimating the practical importance of the present results, it must be noted that the calculations are based on laboratory trials and biomass yields reported in the literature, which have large variation depending on the growing conditions (location, seasonal variations, cultivation practises etc.). Furthermore, the figures presented here for methane potentials per hectare represent the theoretical potential of methane production from energy crops and crop residues, and the energy consumed during production of the biomass and operation of the anaerobic digestion process has not been taken into account when calculating the gross energy potentials. When selecting specific plant species as energy crops for methane production, not only the methane potentials of crops need to be considered, but many other factors, such as the costs and practicability of production and storage of energy crop biomass, are also important in the selection of a feedstock. Thus, all aspects of the production chain, among which the methane potential is a crucial parameter, need to be considered.

It has been found earlier that clonal variations and growing conditions can have a significant effect on the methane potentials (Gunaseelan 1997). Also treatment of plants with various nutrient elements, especially nitrogen, in nutrient-deficient environments during the growth period has been reported to increase methane production (Shiralipour & Smith 1984). Therefore, it is reasonable to assume that the methane potentials of the energy crops could be further increased by using biomass especially bred for the purpose of producing methane and adjusting the nutrient additions with regard to methane production.

5.2 Effect of storage on methane potential of energy crops and crop residues

The present results show that grass and sugar beet tops can be stored as silage at 5 and 20 °C for several months without significant losses in methane potential. Storage of sugar beet tops was shown to be feasible without additives, with only minor (0–13%) losses in methane potential, but with grass, storage without additives led to considerable losses in methane potential (17–39%). Thus, on basis of the present results, the storage of sugar beet tops is likely to be most economical without additives, but with boreal grasses, the need for storage additives in conservation of biomass for methane production is evident (II). Many authors have previously reported various crops stored as silage without additives to have equal or higher methane potentials than fresh crops; for example, with grass and sugar beet tops, increases of 3–19 and 6%, respectively, in methane potential per VS_{added} after storage without additives have been previously reported (Zubr 1986, Chynoweth et al. 1993, Pouech et al. 1998). However, in none of these studies were the losses of VS during storage

considered, whereas the present results show the importance of taking into account the losses of VS when calculating the true methane potential and obtainable energy output (II).

In the present study it was shown that ensiling with additives can be applied as a simultaneous pre-treatment to increase the anaerobic biodegradability of energy crops and crop residues. It has been stated earlier that during ensiling the structural polysaccharides contained in plant material can be partially degraded and intermediates for methanogenic fermentation can be produced (Egg et al. 1993). FA has been reported to degrade plant hemicellulose (Kung et al. 2003) and it has also been applied in delignification of agricultural wastes (Rousu et al. 2002). Addition of cellulose- and hemicellulose-degrading enzymes also has the potential to promote hydrolysis and improve the digestibility of organic matter (Kung et al. 2003). The supernatant of digestate applied in the mixed culture treatment in the present study contains hydrolytic enzymes and bacteria, whereas LAB generally lack the hydrolytic activity towards complex carbohydrates (Rooke & Hatfield 2003). Part of the increase in methane potential per VS_{added} of grass and sugar beet observed after storage with FA, enzymes and mixed culture was probably due to the fact of the additive being used as substrate in methane production. However, the use of these additives improved the conservation of the organic matter during storage, and they were also likely to have increased the anaerobic biodegradability of the crops by partially degrading the recalcitrant plant cell wall fibres, the combined effect of all these factors leading to the observed increase in the methane potential of grass and sugar beet tops after storage with additives. In the present study, storage with the mixed culture obtained from a farm biogas reactor was shown to be a feasible method of conserving the methane potential of grass and sugar beet tops, with potential of simultaneously improving the anaerobic biodegradability of these crops during storage, and, thus, it may offer a cost-efficient solution for biogas-producing farms. However, in the present study the mixed culture was applied in a ratio of 25%, which considerably increases the volume and storage capacity needed, and the application ratio would then need to be optimised further.

The higher losses of organic matter during the storage of sugar beet tops than of grass was apparently partly due to the fact that the sugar beet tops contained more easily degradable compounds compared with grass, as shown by the higher methane potential of fresh sugar beet tops, and these compounds can be more easily lost during sub-optimal storage conditions. Moreover, many storage additives are reported to function better at lower (< 85%) moisture concentrations (McDonald et al. 1991, Woolford 1984, Buxton & O'Kiely 2003), and the high moisture concentration of sugar beet tops (89%) may also have contributed to the high losses of sugar beet top VS during storage with additives.

Storage was shown to promote the solubilisation of organic matter, as indicated by the increase in solubilised nitrogen (NH_4-N) in the stored materials, and to increase the methane production rate from grass. The fact that a short lag in methane production was initially observed in the batch assays with fresh grass indicated that in these assays there were low amounts of soluble organic compounds readily available for methanogens, whereas in the

batch assays with stored materials, such compounds were available immediately and methanogenesis could begin without a lag. The initially low amounts of soluble organic compounds in grass may also have retarded fermentation by the LAB, since they require readily available soluble carbohydrates for fermentation to lactic acid (Rooke & Hatfield 2003), resulting in the high losses of VS observed in the storage of grass with the addition of LAB inoculant. The addition of cell wall degrading enzymes to release additional substrate for LAB is most likely to be beneficial in storage of crops in which the lack of substrate rather than the numbers of viable LAB is the limiting factor in the production of well-preserved silage (Kung et al. 2003). In a combined enzyme treatment and LAB inoculation, enzymes degrade the plant cell walls and thus release intracellular soluble carbohydrates for lactic acid fermentation (Weinberg & Muck 1996). Therefore, this combined treatment could be particularly useful in storage of grass.

With both crops, the extent of solubilisation increased as storage was prolonged, as indicated by the higher shares of NH_4-N from N_{tot} in materials stored for longer periods; this in turn explains the increase in the methane potential per VS_{added} of grass and sugar beet tops as storage was prolonged. However, with grass the increased losses of VS with time overshadowed the increase in methane potential per VS_{added}, and as a result there was little change over time in the overall methane potential of grass stored with additives. However, with sugar beet tops the methane potential per $VS_{original}$ was always higher after storage for 6 months than after storage for 3 months, and therefore storing this material for longer periods, even without additives, is not likely to be detrimental to the conservation of methane potential, and might in fact lead to improvement in the methane potential.

The higher pH of the stored plant biomass, the lower solubilisation rate, as shown by the lower proportion of NH_4-N from N_{tot} in materials stored at 5 °C, and the higher losses in methane potential after storage at 5 °C may have been due to the fact that the temperature of the laboratory silos was lowered to 5 °C immediately after addition of the storage additive, which could have prevented efficient hydrolysis and retarded the growth of LAB. However, in field operation the heat produced by the respiration occurring during the initial aerobic phase of ensiling is likely to ensure higher temperatures in the silos during the beginning of ensiling (Pahlow et al. 2003).

5.3 Anaerobic digestion of crop biomass in CSTR and leach bed processes

5.3.1 Methane yields and post-methanation potentials

The present results show that anaerobic digestion of manure and crops (sugar beet tops, grass silage and oat straw) in CSTRs is feasible with at least up to 40% VS of crops in the feedstock (corresponding to 19, 11 and 4% wet weight of sugar beet tops, grass and straw, respectively) (III). The highest specific methane yields of 0.268, 0.229 and 0.213 m^3 CH_4 kg^{-1} VS_{added} in co-digestion of

cow manure with grass, sugar beet tops and straw, respectively, were obtained during feeding with 30% of crop in the feedstock, corresponding to 85–105% of the total methane potential in the substrates as determined by batch assays. Volumetric methane production increased by up to 65% in reactors fed with 30% VS of crop along with manure, compared with that in reactors fed with manure alone at a similar loading rate. To our knowledge, the present study is the first long-term co-digestion study demonstrating that co-digestion of manure with sugar beet tops and grass is a feasible manner of increasing volumetric methane production without the need to shorten the hydraulic retention time (20 days in the present study) (III). Co-digestion of straws and animal manures has been demonstrated also earlier, but in the present study the increase obtained in specific methane yield after the addition of straw in the feedstock was higher than previously reported (Hashimoto 1983, Fischer et al. 1983, Somayaji & Khanna 1994).

If suitable materials for co-digestion, such as manure, are not available, energy crops can be digested alone in leach bed reactors with or without a second stage methanogenic reactor. Of the leach bed processes included in the present study, the highest methane yields were obtained in the two-stage process without pH adjustment. This process was well suited for anaerobic digestion of the highly degradable sugar beet and grass silage containing 50% clover, yielding 0.382–0.390 m^3 CH_4 kg^{-1} VS_{added} within the 50–55 d solids retention time, corresponding to 85–105% of the total methane potential in the substrates (V). With the more recalcitrant substrates, first year shoots of willow and clover-free grass silage, the methane yields remained at 0.162 and 0.197 m^3 CH_4 kg^{-1} VS_{added}, respectively, corresponding to 59–66% of the total methane potential in substrates (IV–V). As only 20% of the methane potential in grass silage was extracted within the 55 d solids retention time in the one-stage leach bed process, and up to 98% of the total methane yield in the two-stage process originated from the second stage methanogenic reactor, applying a second stage methanogenic reactor in combination with a leach bed process was clearly advantageous.

The methane yields and VS removals obtained in the laboratory two-stage anaerobic digestion process employing batch leach bed reactors in the first stage were of the same order of magnitude as those reported by Yu et al. (2002), who obtained a 0.165 m^3 CH_4 kg^{-1} VS_{added} methane yield and 67% VS removal, and Cirne et al. (2006), who reported a 0.27 m^3 CH_4 kg^{-1} VS_{added} methane yield and 60% VS removal, in laboratory batch leach bed processes connected to anaerobic filters, digesting grass waste (Yu et al. 2002) and grass silage (Cirne et al. 2006). However, the obtainable methane yields in one- and two-stage leach bed processes with clover-free grass silage (IV) were low compared with those obtained in CSTRs co-digesting similar grass silage with cow manure (III). The higher specific methane yields obtained in co-digestion of grass silage and cow manure compared with digestion of grass silage in leach bed processes or digestion of manure alone in CSTRs may also be due to synergy effects owing to a more balanced nutrient composition and C/N ratio in the co-digestion feedstock. This is also supported by the fact that in CSTRs with 20 d HRT, high proportions of up to 131 and 105% of the methane potentials measured in the methane potential assays after 20 and 80–100 days, respectively, were obtained.

Furthermore, in manure, which has already passed through the digestive track of the animal, most of the energy-rich substances (*i.e.* carbohydrates and proteins) contained in the crops, have already been digested. The high methane yields obtained in co-digestion can also be partly explained by microbial adaptation, which is likely to be enforced by the semi-continuous feeding in CSTRs, as opposed to the situation in batch reactors. Furthermore, in the two-stage processes applied in the present study, no inoculum addition was done in the first stage. Inoculating the batch reactors with digestate from previous runs would enable continuous adaptation of microbes to the degradation of the substrate and would be likely to enhance the extent of degradation and methane production also in batch processes.

The benefits of optimising the proportion of crops and loading rate in co-digestion were shown by the fact that during feeding with 30% VS of crop in the feedstock, up to 87% higher specific methane yield was obtained than with the lower proportions of crop, while increasing the proportion of crop further (to 40%) led to a decrease of up to 12% in specific methane yields. Furthermore, the highest specific methane yields were obtained at the OLR of 2 kg VS m^{-3} d^{-1} with 20 HRT, while increasing the OLR and decreasing the HRT (from 20 to 16 days) led to a 16–26% decrease in specific methane yield. At the higher OLRs, volumetric methane production increased, but the retention times apparently became too short for efficient degradation, as the amounts of undegraded matter in the digestates increased, leading to an increase in the post-methanation potentials. Also in co-digestion of pig manure and potato waste in laboratory CSTRs with 0, 15 and 20% VS of potato waste in the feedstock, increasing the OLR from 2 to 3 kg VS m^3 d^{-1} resulted in a 7–15% decrease in specific methane yield, while the highest specific methane yields were obtained with the 20% proportion of potato waste (Kaparaju & Rintala 2005), and in laboratory digesters fed daily with manure and wheat straw, with 0–100% TS of wheat straw in the feedstock, the highest specific methane yields were observed with 40% wheat straw, whereas highest VS removal was obtained with 20% wheat straw (Somayaji & Khanna 1994). However, neither of these experiments included feedstock with 30% of crop material, which was found optimal in the present study.

Post-methanation of digestates sampled from CSTRs during co-digestion of manure and crops indicated that the digestates still contained degradable material with significant methane potential, which, if completely recovered, would in northern climatic conditions correspond to 0.9–2.5 m^3 CH_4 t^{-1} ww of digestate (calculated assuming post-methanation potential at 20 and 5 °C each for 6 months of a year) and up to 12–31% of total methane production (sum of methane production in reactors and in post-methanation), being highest following co-digestion of manure with straw. If not recovered, part of this post-methanation potential may be lost as emissions to the atmosphere. In contrast, if the post-storage tanks were maintained at 20 or 35 °C throughout the year, a post-methanation potential of 1.7–4.7 or 2.9–7.7 m^3 CH_4 t^{-1} ww of digestate, respectively, could be obtainable, corresponding to 21–43 and 35–56% of total methane production. According to Kaparaju & Rintala (2003), digested cow manure had a post-methanation potential of 0.206–0.240 m^3 CH_4 kg^{-1} VS_{added} after 250 days at 35 °C, whereas at 20 and 5 °C it amounted to 0.087–0.088 and

0.003–0.005 m^3 CH_4 kg^{-1} VS_{added}, respectively. These values are of the same order of magnitude as those obtained in the present study, and thus, co-digestion of crop materials with manure would not seem to significantly enhance the post-methanation potential of the digestates. The post-methanation potentials of digestates from anaerobic digestion of grass silage in leach bed processes corresponded to 19–22 m^3 CH_4 t^{-1} ww of digestate (measured at 35 °C) and 42–93% of the total methane production, the proportion being highest after digestion of grass in the one-stage leach bed process. The post-methanation potentials of digestates from the leach bed processes were of the same order of magnitude as those from CSTRs when calculated per VS of digestate, but owing to the high TS concentrations, an order of magnitude higher than those obtained in post-methanation of digestates from CSTRs.

5.3.2 Operation of the CSTRs and leach bed processes

In all the CSTRs, the accumulation of undegraded material was observed as the formation of a crust on the upper part of the liquid space, and was most apparent in co-digestion of straw and manure. The formation of crust did not cause problems in laboratory operation, but in full scale operation it might have more serious outcomes, such as fouling in gas collection pipes, scum overflow, and thermal stratification (Hobson & Wheatley 1993). It has been reported previously that crust formation in co-digestion of crops and manure at a TS concentration of about 10% could be prevented by a sufficient reduction in the particle size of crop materials along with continuous stirring (Nordberg & Edström 1997).

In the one-stage leach bed process, 80% of the grass SCOD (as determined after 24 h extraction) was extracted within the 55 d retention time, whereas the corresponding figure for the two-stage anaerobic digestion process employing a batch leach bed reactor and UASB, without pH adjustment, was 241%. In the one-stage operation, 83% of the extracted COD was converted to methane, whereas the corresponding figure for the two-stage operation was 92–95%. The low COD extraction rate in the one-stage operation was apparently due to the high SCOD and VFA concentrations in the recirculated leachate (SCOD and TVFA up to 15 and 7 g l^{-1}, respectively), which can cause inhibition of hydrolysis and acidogenesis (Vavilin et al. 2003), whereas in the two-stage operation, the UASB efficiently removed SCOD and VFA from the leachate (up to 99% SCOD reduction), as a result of which the UASB effluent returned to the batch leach bed reactor was low in SCOD and VFA (mostly < 1 g l^{-1}), resulting in turn in more efficient extraction of grass SCOD. VFA accumulation was apparently the cause of the lower methane yield and lower VS removal also in run 4 with six parallel leach bed reactors, where the lower L/S ratio (8) applied resulted in higher SCOD and VFA concentrations in the leachate as opposed to the corresponding run with a L/S ratio twice as high (17 in run 2).

In total, 39% of the carbohydrates were removed in the leach bed - UASB process within the 49 days of operation. Proteins were the most rapidly hydrolysable component in grass, as they were degraded to the highest extent after 1 day of liquid recirculation, whereas extractives were the most solubilised component after 10 and 49 days of operation. The apparent loss of lignin in

leach bed digesters fed with grass silage was most probably due to solubilisation rather than degradation, as also suggested by Kivaisi et al. (1990), as lignin is known to be refractory and poorly degraded in anaerobic conditions (Fan et al. 1981). However, in the present study it was shown that more than half of the acid soluble lignin was solubilised after 49 days of digestion in a leach bed digester fed with grass silage, whereas Klason lignin was the most recalcitrant component of those determined in the present study.

In laboratory two-stage operation, adjustment of the pH of influent to the leach bed reactor to 6 with HCl led to inhibition in both the leach bed reactor and the UASB. Inhibition of hydrolysis and acidogenesis in the leach bed process were indicated by the low SCOD values and the low share of TVFA of SCOD in the leachate, whereas inhibition of methanogenesis in the UASB was indicated by the presence of VFAs in the UASB effluent and by the lower and fluctuating methane concentration in the gas from the UASB (varying between 46 and 60%) compared with that in the corresponding run without pH adjustment, despite the similar UASB loading rates (5 kg COD m^{-3} d^{-1}) in the two experiments. The low VS removal and the high post-methanation potential of digestate from the run with pH adjustment indicated a much lower extent of degradation than in the corresponding experiment without pH adjustment, with the result that the total specific methane yield from the run with pH adjustment remained much lower than in the corresponding run without pH adjustment despite the similar UASB loading rates. Due to the problems in the UASB, the run with pH adjustment was terminated after only 31 days of operation. pH values of around 6 have been reported optimal for the functioning of the extracellular cellulase enzymes produced by hydrolytic bacteria (Sleat & Mah 1987), and therefore it was assumed that pH adjustment to 6 could be advantageous in a leach bed process. However, debate has arisen about the optimum values of pH for hydrolysis, and some authors have claimed that lowering the pH below neutral would not clearly enhance the rate of hydrolysis (Veeken et al. 2000, Dinamarca et al. 2003, Babel et al. 2004). The present results support this hypothesis. Moreover, chloride ion has been reported to give rise to toxic effects in anaerobic wastewater treatment (Mendez et al. 1992, Vijayaraghavan & Ramanujam 1999), and thus it is possible that the low methane yields and VS removal in run 3 were caused by inhibitory effects due to the application of HCl in pH adjustment. However, Wujcik and Jewell (1980) found no inhibitory effect due to increased chloride concentrations (added as NaCl) in high solid digesters digesting newsprint paper and dairy manure, and Zhang et al. (2005) did not report any inhibitory effects in hydrolysis and acidogenesis of kitchen waste when HCl was used in pH adjustment.

In pilot experiments, the MF with digested straw as carrier material had been in operation for about two years before initiation of these experiments, and the earlier results with this reactor show similar and even superior performance over the MF with plastic carriers (Parawira et al. 2006). It is possible that the straw bed aging could have caused a collapse in the structure or that channelling could have occurred. Biological materials such as straw are cheap to use as a carrier material, but the risk of unreliable performance due to aging and need for periodic renewal and consequent long start-up periods are

disadvantages, making plastic carriers more reliable. Furthermore, the loading rates to the MFs in these set-ups were manually controlled, and thus relatively difficult to maintain. Consequently, automatic control may be necessary in order to make the systems more stable and easier to operate. For example, on-line monitoring of biological oxygen demand (BOD) or VFAs have been suggested as control strategies for two-stage processes (Sachs et al. 2003, Liu et al. 2004).

The inoculation ratio applied in the one-stage leach bed processes digesting grass silage (6% of inoculum of total VS) was apparently too low for an efficient utilisation of the methane potential in the substrate, as indicated by the low specific methane yield, low VS removal, and high post-methanation potential in the digestate. Torres-Castillo et al. (1995) studied digestion of barley straw in batch leach bed reactors with varying inoculum concentrations (2–12% of VS), and the highest gas production was obtained in the reactor where the share of inoculum was highest (12% of VS: 0.226 m^3 CH_4 kg^{-1} VS_{added}). However, the difference in gas production between the reactors inoculated with 12 and 6% of inoculum (0.211 m^3 CH_4 kg^{-1} VS_{added}) was only minor and overshadowed by the lower volumetric gas production at the higher inoculum application ratios. Therefore, the authors recommended the use of 6% of inoculum of total VS (Torres-Castillo et al. 1995). In digestion of wheat straw in batch leach bed reactors with varying inoculum concentrations (5 to 20% of inoculum of total VS), the difference in reactor performance using a large or small addition of inoculum was insignificant after a few days of hydrolysis, and an inoculum concentration of up to 5% was suggested sufficient for a proper start-up (Llabrés-Luengo & Mata-Alvarez 1988). However, grass silage is a more biodegradable substrate than straw, as indicated by the higher methane potential and the higher amounts of readily available soluble compounds in grass compared with straw (I, III). Therefore, the inoculation ratio previously recommended for the digestion of straw may have been too low for that of grass silage, which points to the need to optimise the substrate/inoculum ratios for batch processes digesting energy crops.

Leachates at the end of digestion of sugar beet and grass-clover silage in pilot reactors were well suited as nitrogen-rich irrigation water, having high concentrations of mineralised nitrogen (NH_4-N). As digestates with C/N ratios of around 10 are suitable for incorporation to soil as soil-improvement media (Demuynck 1984), the solid residues from digestion of sugar beet and grass were well suited for this purpose. However, in the solid residue from digestion of willow, cadmium concentration exceeded the limit value for use of digestates as fertiliser (Swedish EPA 2005). It was shown that the heavy metals were strongly mobilised during the acidic phase, which could offer a possibility of precipitating metals from the leachate during the acidic phase and thus removing the metals from the farming system. This option is especially interesting in areas like Southern Sweden, where cadmium concentrations in arable land are high due to anthropogenic inflows (land application of inorganic fertilisers and atmospheric deposition) (Swedish EPA 2005). Cultivation of crops with the ability to accumulate more cadmium than most agricultural crops has been suggested for phytoextraction of metals from arable land (Berndes et al. 2004). Consequent two-stage anaerobic digestion of these

crops with simultaneous removal of heavy metals from leachates, e.g. through biological precipitation by sulphate-reducing bacteria (Möller et al. 2004), could offer possibilities for remediating polluted soils.

6 CONCLUSIONS

The results presented here identify a large reservoir of boreal plant species that can be harnessed in methane production. The results show that up to 3 000–5 000 m^3 of methane and 30–50 MWh of gross energy can potentially be obtained from one hectare of energy crops cultivated for biogas production. Most crops were shown to have methane potentials in the range 0.3–0.4 m^3 CH_4 kg^{-1} VS_{added}. The methane potentials per ww increased with most crops as the crops matured, and as the biomass yield also mostly increases as the harvest is postponed, the later harvest would be more optimal in most cases.

The present results show that storage of sugar beet tops for methane production is likely to be feasible without any storage additives, while with grass, storage without additives will lead to considerable losses of methane potential. Furthermore, ensiling with additives was shown to have potential in improving the methane potentials of these substrates, and thus, storage can be applied as a simultaneous pre-treatment to increase the methane potential of energy crops and crop residues. The duration and temperature of storage had little influence on the methane potential of grass stored with additives, whereas that of sugar beet tops increased as storage was prolonged and was better conserved at 20 °C. Storage with the mixed culture obtained from a farm biogas reactor was shown to be a feasible method of conserving methane potential in grass and sugar beet tops, and, thus, it may offer a cost-efficient solution for biogas-producing farms.

Anaerobic digestion of cow manure and crops (sugar beet tops, grass silage and oat straw) in CSTRs was shown feasible with up to 40% VS of crops in the feedstock. The highest specific methane yields of 0.268, 0.229 and 0.213 m^3 CH_4 kg^{-1} VS_{added} in co-digestion of cow manure with grass, sugar beet tops and straw, respectively, were obtained during feeding with 30% of crop in the feedstock, corresponding to 85–105% of the total methane potential in the substrates as determined by batch assays. Volumetric methane production increased by up to 65% in reactors fed with 30% VS of crop along with manure, compared with that in reactors fed with manure alone at a similar loading rate. After doubling the OLR from 2 to 4 kg VS m^{-3} d^{-1} less methane was extracted

per added VS, leading to a 16–26% decrease in specific methane yields, thus leaving more untapped methane potential being left in the residues. The post-methanation potential of the digestates, if completely recovered, would in northern climatic conditions correspond to 0.9–2.5 m^3 CH_4 t^{-1} ww of digestate and up to 12–31% of total methane production, the highest levels following co-digestion of manure with straw. If the post-storage tanks were maintained at 20 or 35 °C throughout the year, a post-methanation potential of 1.7–4.7 or 2.9–7.7 m^3 CH_4 t^{-1} ww of CSTR digestate, respectively, could be obtainable, corresponding to 21–43 and 35–56% of total methane production.

If suitable materials for co-digestion, such as manure, are not available, energy crops can be digested alone in leach bed reactors with or without a second stage methanogenic reactor. Of the leach bed processes included in the present study, the highest methane yields were obtained in the two-stage process without pH adjustment. This process was well suited for anaerobic digestion of the highly degradable sugar beet and grass-clover silage, yielding 0.382–0.390 m^3 CH_4 kg^{-1} $VS_{added,}$ corresponding to 85–105% of the total methane potential in the substrates. With the more recalcitrant substrates, first year shoots of willow and clover-free grass silage, the methane yields remained at 0.162 and 0.197 m^3 CH_4 kg^{-1} VS_{added}, corresponding to 59–66% of the total methane potential in substrates. As only 20% of the methane potential in grass silage was extracted within the 55 d solids retention time in the one-stage leach bed process, and up to 98% of the total methane yield in the two-stage process originated from the second stage methanogenic reactor, applying a second stage methanogenic reactor in combination with a leach bed process was clearly advantageous. In the two-stage operation, adjustment of the pH of influent to the leach bed reactor to 6 with HCl inhibited both hydrolysis/acidification and methanogenesis. In the leach bed-UASB process about 39% of the carbohydrates in clover-free grass silage were removed and more than half of the acid soluble lignin was solubilised, whereas Klason lignin was the most recalcitrant component of those determined in the present study. The digestates from digestion of clover-free grass silage in the leach bed processes still contained degradable material with significant methane potential, which, if completely recovered, would correspond to 0.141–0.204 m^3 CH_4 kg^{-1} VS_{added} and 19–22 m^3 CH_4 t^{-1} ww of digestate (at 35 °C). Liquid and solid residues from digestion of grass-clover silage and sugar beet in the leach bed – MF processes were suitable for incorporation to soil as fertiliser and soil-improvement media, whereas in the solid residue from digestion of willow, cadmium concentration exceeded the limit value for use of digestates as fertiliser. Efficient mobilisation of heavy metals during the acidic phase of digestion revealed the possibility of removing (precipitating) metals from the leachate generated in two-stage anaerobic digestion of phytoextracting crops, thus remediating contaminated soils and removing metals from a farming system.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Energiakasvien ja kasvijätteiden hyödyntäminen biokaasun tuotannossa

Peltobiomassat, sekä energiantuotantoa varten viljellyt energiakasvit että erilaiset kasvintuotannon sivutuotteet ja jätteet, tarjoavat hiilidioksidineutraalin, joustavan ja kotimaisen uusiutuvan energialähteen. Kasvibiomassa voidaan hyödyntää energiana useilla eri tavoilla, joista yksi on biokaasun tuotanto. Biokaasureaktorissa mikrobit hajottavat hapettomissa olosuhteissa orgaanista ainesta, ja hajotuksen lopputuotteena syntyy runsaasti metaania sisältävää biokaasua, joka voidaan hyödyntää lämmön- ja/tai sähköntuotannossa tai liikenteen polttoaineena fossiilisten polttoaineiden sijasta.

Tässä väitöstyössä tutkittiin mahdollisuuksia hyödyntää energiakasveja ja kasvijätteitä biokaasun tuotannossa pohjoisissa oloissa. Tutkimuksessa kartoitettiin Suomen oloissa menestyviä, biokaasun tuotantoon mahdollisesti soveltuvia kasvilajeja, sekä määritettiin niiden kemiallinen koostumus ja metaanintuottopotentiaali. Lisäksi tutkittiin korjuuajankohdan ja eri varastointimenetelmien vaikutuksia kasvien metaanintuottopotentiaaliin sekä erilaisten reaktoritekniikoiden soveltuvuutta kasvibiomassan anaerobiseen käsittelyyn.

Kasvien metaanintuottopotentiaalit vaihtelivat välillä 0,17–0,49 m^3 CH_4 kg^{-1} $VS_{lisätty}$ (lisättyä orgaanista ainetta kohti) ja 25–260 m^3 CH_4 tonnia (märkäpaino) kohti, ollen useimmilla kasveilla välillä 0,3–0,4 m^3 CH_4 kg^{-1} $VS_{lisätty}$. Tutkituista kasveista maa-artisokalla, timotei-apilanurmella ja ruokohelpillä oli korkein metaanintuottopotentiaali hehtaaria kohti, 2 900–5 400 m^3 CH_4 ha^{-1} (hehtaari), mikä vastaa 28–53 MWh ha^{-1} bruttoenergiasaantoa. Mikäli tämä metaanimäärä hyödynnettäisiin henkilöautojen polttoaineena, riittäisi hehtaarilta saatava metaani 40 000–60 000 ajokilometriin vuosittain. Korjuuajankohdan vaikutus kasvien metaanintuottopotentiaaliin orgaanista ainetta kohti vaihteli paljon eri kasveilla, mutta useimpien kasvien metaanintuottopotentiaali märkäpainotonnia kohti kasvoi, kun korjuuta lykättiin myöhemmäksi.

Kun nurmiheinää ja sokerijuurikkaan naatteja varastoitiin säilörehuntekomenetelmällä laboratoriosiiloissa ilman varastointilisäaineita, menetettiin 3–6 kuukauden varastoinnin aikana 17–39 % nurmiheinän metaanintuottopotentiaalista, mutta sokerijuurikkaan naateilla metaanintuottopotentiaalin häviöt olivat vähäiset (0–13 %). Siten metaanintuottoa varten korjattujen sokerijuurikkaan naattien varastointi on todennäköisesti kannattavinta ilman varastointilisäaineita, mutta nurmiheinän varastoinnissa ne ovat tarpeen varastointihäviöiden pienentämiseksi. Varastoimalla nurmiheinää ja sokerijuurikkaan naatteja säilörehuntekomenetelmällä varastointilisäaineiden kanssa voitiin näiden kasvien metaanintuottopotentiaalia parantaa korkeimmillaan 19–22 % verrattuna tuoreiden kasvien metaanintuottopotentiaaliin. Varastoinnin kestolla ja lämpötilalla oli vain vähän vaikutusta lisäaineiden kanssa varastoidun nurmiheinän metaanintuottopotentiaalin säilymiseen, kun taas sokerijuurikkaan naattien metaanintuottopotentiaali säilyi paremmin 20 °C:ssa kuin 5 °C:ssa ja kohosi va-

rastoinnin pitkittyessä. Nurmiheinän ja sokerijuurikkaan naattien varastointi biokaasureaktorista peräisin olevan mikrobisiirroksen kanssa osoittautui tehokkaaksi varastointimenetelmäksi, joten se voisi olla edullinen vaihtoehto näiden materiaalien varastointiin maatilakohtaisten biokaasulaitosten yhteydessä.

Energiakasvien (säilörehu) ja kasvijätteiden (sokerijuurikkaan naatit, kauran olki) yhteiskäsittelyä lehmänlannan kanssa tutkittiin laboratoriomittakaavan täyssekoitteisissa lietereaktoreissa lisäämällä kasvien määrää syötteessä vähitellen nollasta 40 prosenttiin syötteen orgaanisesta aineesta. Korkeimmat metaanisaannot (0,268 m^3 CH_4 kg^{-1} $VS_{lisätty}$ heinän, 0,229 m^3 CH_4 kg^{-1} $VS_{lisätty}$ sokerijuurikkaan naattien ja 0,213 m^3 CH_4 kg^{-1} $VS_{lisätty}$ olkien yhteiskäsittelyssä lannan kanssa) saatiin, kun kasvin osuus syötteen orgaanisesta aineesta oli 30 % ja reaktorien kuormitus 2 kg VS m^{-3} d^{-1}. Nämä metaanisaannot vastasivat 85–105 % käsiteltyjen materiaalien metaanintuottopotentiaalista. Kasvien lisääminen syötteeseen (30 % VS:sta) lisäsi reaktorien kaasuntuottoa reaktoritilavuutta kohti enimmillään 65 % verrattuna pelkän lannan käsittelyyn vastaavissa olosuhteissa. Reaktorien kuormituksen kaksinkertaistaminen kahdesta neljään kg VS m^{-3} d^{-1} kasvatti edelleen kaasuntuottoa reaktoritilavuutta kohti, mutta laski ominaismetaanintuottoa 16–26 % ja kasvatti vastaavasti käsitellyn materiaalin jälkikaasutuspotentiaalia. Käsitellyn materiaalin jälkikaasutuspotentiaali vastasi pohjoisissa oloissa 0,9–2,5 m^3 CH_4 tonnia käsiteltyä materiaalia kohti, ollen korkeimmillaan oljen ja lannan yhteiskäsittelyn jälkeen. Mikäli jälkikaasutussäiliöiden lämpötila pidettäisiin läpi vuoden 35 °C:ssa, olisi jälkikaasutuksessa mahdollista saavuttaa metaanintuotto, joka vastaisi 2,9–7,7 m^3 CH_4 tonnia käsiteltyä materiaalia kohti.

Mikäli soveltuvia materiaaleja, kuten eläinten lantaa, ei ole saatavilla yhteiskäsittelyyn, voidaan pelkkää kasvibiomassaa käsitellä esimerkiksi panosperiaatteella toimivissa suotopetireaktoreissa, joita voidaan operoida yhdessä erillisten metaanintuottoreaktorien kanssa. Tässä tutkimuksessa vertailtiin laboratoriokokeissa timoteivaltaisen säilörehun käsittelyä yksivaiheisessa suotopetireaktorissa, ja suotopetireaktorin ja UASB-reaktorin (upflow anaerobic sludge blanket) yhdistelmässä, sekä pilot-mittakaavan kokeissa yksivuotisen pajun, apilavaltaisen säilörehun ja sokerijuurikkaiden (juurikkaat ja naatit) käsittelyä suotopetireaktorien ja anaerobisten suotimien yhdistelmässä. Viimeksi mainittu prosessi soveltui hyvin apilavaltaisen säilörehun ja sokerijuurikkaiden käsittelyyn, sillä niiden metaanintuottopotentiaalista saavutettiin tässä prosessissa 85–105 %, eli prosessin metaanintuotto vastasi 0,382–0,390 m^3 CH_4 kg^{-1} $VS_{lisätty}$, kun taas metaanisaannot pajun ja timoteivaltaisen säilörehun käsittelyssä näissä prosesseissa jäivät alhaiseksi, 0,162–0,197 m^3 CH_4 kg^{-1} $VS_{lisätty}$, vastaten 59–66 % näiden materiaalien metaanintuottopotentiaalista. Yksivaiheisessa suotopetireaktorissa saavutettiin vain 20 % timoteivaltaisen säilörehun metaanintuottopotentiaalista 55 päivän aikana, kun taas kaksivaiheisissa prosesseissa enimmillään 98 % metaanisaannosta oli peräisin toisen vaiheen metaanireaktorista. Kaksivaiheisessa prosessissa 39 % timoteivaltaisen säilörehun hiilihydraateista ja yli puolet happoliukoisesta ligniinistä liukeni, kun taas Klason-ligniini osoittautui määritetyistä yhdisteistä vaikeimmin anaerobisissa oloissa hajoavaksi.

Timoteivaltaista säilörehua käsittelevissä suotopetiprosesseissa käsitellyn materiaalin jälkikaasutuspotentiaali oli 0,141–0,204 m^3 CH_4 kg^{-1} $VS_{lisätty}$ ja 19–22 m^3 CH_4 tonnia käsiteltyä materiaalia kohti 35 °C:ssa mitattuna. Sekä nestemäisessä että kiinteässä fraktiossa olevat jäännökset säilörehun ja sokerijuurikkaan käsittelyn päätteeksi soveltuivat hyvin käytettäviksi lannoitteena ja maanparannusaineena, kun taas pajun käsittelyssä kiinteän jäännöksen kadmiumpitoisuudet ylittivät peltolevitykselle asetetut raja-arvot. Kaksivaiheisen prosessin happovaiheessa raskasmetallit liukenivat tehokkaasti prosessin kiertoveteen, mikä voisi mahdollistaa prosessin hyödyntämisen raskasmetallien poistoon maaperästä fytoekstraktioon, eli kasveilla tapahtuvaan haitta-aineiden poistoon maaperästä, sopivien kasvilajien avulla.

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